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# ENTOMON

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# ENTOMON

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## *Short Commuinication*

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# Bees of the genera *Hoplonomia and Crocisaspidia* (Halictidae, Nomiinae) of India, with key to species

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**ABSTRACT:** The nomiine bees of the genera *Hoplonomia* Ashmead and *Crocisaspidia* Ashmead occurring in India are reviewed. Specimens were collected from Kerala. Biology and distribution of five species are summarized. An identification key to *Hoplonomia and Crocisaspidia* of India is provided.


**KEY WORDS:** Hymenoptera, Apoidea, taxonomy, distribution

## INTRODUCTION

*Hoplonomia* Ashmead, 1904 is mainly a south Asian genus occurring from Afghanistan to Australia. It is not known from Africa (Michener, 2007). This genus is recognized by the banded abdomen and basally fused lamelliform projections on the metanotum. *Hoplonomia* is considered intermediate between *Acunomia* and *Crocisaspidia. Hoplonomia* is represented by 16 species worldwide. Five species *viz, Hoplonomia elliotii* (Smith, 1875), *H. kulliensis* (Tomar & Tomar, 2005)*, H. westwoodi* (Gribodo, 1894), *H. callichora* (Cockerell, 1911) *and H. incerta* (Gribodo, 1894) are known from India. *Crocisaspidia* Ashmead, 1899 is characterized by the presence of double projection of scutellum and metanotum. The body is more robust than in most nomiine genera and is the only group of Nomiinae in which the coloured tergal bands are sometimes broken medially and these broken bands resemble *Thyreus.* Currently a single species is known from India. Eleven species were revised by Pauly (1990). In this paper four species of *Hoplonomia viz*., *H. elliotii*, *H. incerta, H. westwoodi, and H. callichlora* and one species of *Crocisaspidia viz., C. buddha* (Westwood, 1875) are diagnosed. *Hoplonomia kulliensis* probably a synonym of a species already described and being a doubtful species is not included here for diagnosis Pauly (2009).

## MATERIALS AND METHODS

Literature was surveyed for published reports of *Hoplonomia* in India (Bingham, 1897; Pauly, 2009; Saini and Rathore, 2012; Pannure and Belvadi, 2017). Data regarding specimens from the six major collections for India *viz.*, American museum of natural history(AMNH), Regional Museum of Natural History (RMNH), United States National Museum (USNM), Smithsonian Institution, Washington, Statens Museum for Kunst (SMUK) Denmark, Natural History Museum (NHMUK),

* Author for correspondence

Institut royal des Sciences naturelles de Belgium (IRSNB); and Zoological Museum, University of Copenhagen (ZMUC) were obtained. Materials were also collected from the state of Kerala, India using sweep nets. These materials were examined and preserved in the Department of Zoology, Government College Kodanchery, Kozhikode, Kerala, India (DZGCK). Distribution maps were prepared using all localities from India from where *Hoplonomia* and *Crocisaspidia* have been collected and was prepared using SimpleMappr (Shorthouse, 2010).

## RESULTS AND DISCUSSION

Systematics

Halictidae, Nomiinae

Genus *Hoplonomia* Ashmead

Description: Length 6.5 to 11.5mm. Metanotum with two broad, basally fused lamelliform projections as in *Crocisasipidia*. Scutellum lacks projections or has only small ones in some males. Body is generally smaller and slender when compared to *Crocisasipidia.* Intermediate tibia with strong sub apical teeth. Propodeum sub vertical, defined, triangular propodeal area and tegulae normal. Clypeus in the form of plateau, flanked on the front with a median carina more or less marked.

Presence of two lamelliform projections on the metanotum is a characteristic feature which distinguishes *Hoplonomia* from other Nomiinae. This genus is allied to *Curvinomia* Michener on account of the abdomen being banded. Post scutellum in both sexes is armed with two straight spines. Scutellum provided with median depression and the hind angles ending in a small tubercle.

Distribution: *Hoplonomia* is known from Japan, China, India, South East Asia, Philippines, Indonesia, New Guinea, Bismarck, Solomon Islands, Australia, south to southern Queensland and Madagascar. It is not known from Africa. In India it is known from Maharashtra, Himachal Pradesh, Kerala, Karnataka, West Bengal, Andhra Pradesh, Pondicherry, Tamil Nadu and Rajasthan.

**1. *Hoplonomia callichlora* (Cockerell, 1911)**

*Nomia callichlora* Cockerell, 1911: 219, ♀. Holotype ♀: "N.W. India" [Pakistan], Karachi, leg. E. Comber, NHMUK (examined).

Distribution: North India (Fig. 26)

Materials Examined: Rajasthan, Abu, 24.64N; 72.77E, 1 ♀, leg. C.G. Nurse (NHMUK).

Diagnosis: *Length,* 9mm. Black, abdomen with broad bright green bands (the first three flushed with vermillion) on the first four segments; post scutellum with two strong black teeth; mandibles dark red in middle; flagellum ferruginous beneath; hind legs almost entirely amber colored; middle of face and clypeus with a delicate keel; tergite band covers the entire apical depression. *Male*. Femur less developed. *Female*. scutum with more patchy punctuation.

Shows much resemblance to *Hoplonomia elliotii* Smith, it is however readily separated as follows. Rather smaller, face narrower, covered with white hair; mesothorax smaller, more shining, with smaller punctures, a band of grayish-white hair running along lateral and hind margins, and the disc with much pale hair, hair of outer side of middle tibiae entirely white, green abdominal bands twice as broad.

**2. *Hoplonomia elliotii* Smith, 1875**

*Nomia elliotii* Smith, 1853:89, ♂, India Nomen nudum.

*Nomia elliotii* Smith*,* 1875*:*44, pl. 1, fig. 7, ♀♂

Types: Madras, Barrackpore and Nischiudipore. (not examined)

*=? Nomia simplicipes* Friese 1897: 73, ♂, nec. ♀ Lectotype ♂: China, Kaulun near Hong Kong designated by Cockerell, 1919: 3 (not examined) Syn.of Pauly (2009)

Distribution: The species is known from India to Indochina and Southern China. In India it has been reported from Maharashtra, Karnataka, Kerala, Tamil Nadu, West Bengal, Goa and Assam (Fig. 25)

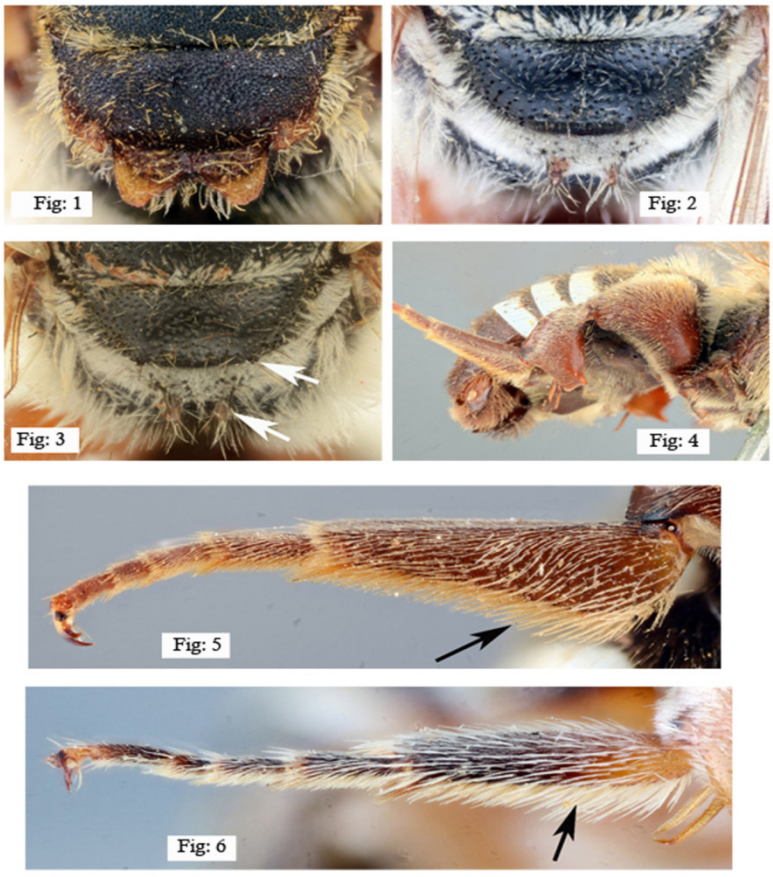


Fig. 1 *Crocisaspidia buddha*, male, scutellum and metanotum, Fig. 2 *Hoplonomia westwoodi,* male, scutellum and metanotum, Fig. 3 *Hoplonomia westwoodi,* scutellum and metanotum, Fig. 4 Hind leg of *Crocisaspidia buddha,* Fig. 5 Enlarged metabasitarsus of *Crocisaspidia,* Fig. 6 Narrow metabasitarsus of *Hoplonomia*

Materials Examined**:** Kerala, Malappuram, Calicut University, botanical garden, 11.13N; 75.89E, 27. ix.1986, 2 ♂, leg. E.E. Grissell (USNM); Trivandrum, Ponmudi Range, 8.48N; 76.94E, 3000ft, v.1972, 2♀, leg. T.R.S. Nathan (SMUK); Palakkad, Walayar Forest, 10.84N; 76.84E, 700ft, x.1959, 4 ♂, leg. P.S. Nathan (RMNH); Palakkad, Walayar Forest, 10.84N; 76.84E, 1000 ft, ix.1952, 1♀, leg. P.S. Nathan (IRSNB); Kasaragod, Neeleswaram, 12.25N; 75.12E, 24.vi.2018, 1♀, leg. Asha (DZGCK); Wayanad, Pulpally, 11.79N 76.16E, 13.v.2018, 1♀, leg. Manjusha (DZGCK); Kasargod, Periye, 12.40N; 75.09E, 10.i.2015, 1♀, leg. Sangeetha (DZGCK); Kozhikode, Balussery, 11.45N; 75.82E, 23. v. 2018, 1♀leg. Adersh (DZGCK); Kannur railway, 11.87N; 75.36E, 6. vii. 2014, 1♀, leg. Asha (DZGCK); Kasaragod, Padannakkad, 12.26N; 75.36E, 2. v. 2018, 1♀leg. Adarsh (DZGCK); Kannur, Madayipara, 38m, 12.16N; 75.33E, 1♂, 13. viii. 2015, coll. Prashantha, C, 1♀, 13. viii. 2015, coll. Pradeepa, S.D.

Maharashtra, Salsette Island, 19.11N 72.89E, 1910, 2♀, (NHMUK).

Tamil Nadu, Coimbatore, 11.00N 76.96E, xi.1958, 1♀ leg. P.S. Nathan (RMNH); Annamalai Hills,11.11N 77335E Cinchona, 3500ft, v.1960, 1♀,leg. P.S. Nathan (RMNH); Nilgiris Hills, Devala, 11.47N; 76.38E, 3200 ft, v.1961, 1♀ leg. P.S. Nathan (AMNH).

West Bengal, Calcutta, 22.57N; 88.36E 1♂, 1♀ (IRSNB).


Fig. 7


Fig. 8


Fig. 9


Fig. 10


Fig. 11


Fig. 12

Fig. 7 *Hoplonomia incerta,* male, metasoma, Fig. 8 *Hoplonomia elliotii, male, metasoma,* Fig. 9 *Hoplonomia westwoodi,* male, *metasoma,* Fig. 10 *Hoplonomia elliotii,* male, hind leg, Fig. 11 *Hoplonomia westwoodi,* male, hind leg, Fig. 12 *Crocisaspidia buddha*, female, scutellum and metanotum

Diagnosis: *Female. Colouration.* Head and mesosoma black, bright emerald green transverse fasciae in the apical margin of the basal four abdominal segments, post scutellar spine dark chestnut brown. *Sculpture.* Head and thorax closely and finely punctured, punctures more sparsely and coarsely on scutellum, smooth and opaque abdomen, punctures on the base of the segments, clypeus flat with carina in the mid line, scutellum with deep notch, presence of spines in the middle of post scutellum posteriorly, fine punctures in the space at the base of the median segment, transverse impressed lines across the middle of the basal four abdominal segments. *Pubescence*- white somewhat griseous thin pubescence, legs with pale glittering pubescence.

*Male.* Tubercles at the lateral angles of the scutellum and medial spines on the post scutellum more prominent, posterior femur greatly swollen, tibia flattened, the apex on the inner side produced into blunt testaceous process.

### 3. *Hoplonomia incerta* (Gribodo, 1894)

*Nomia incerta* Gribodo 1894: 129, ♂, ♀, Lectotype ♀: Java, collection Gribodo, MCSN (examine), designated by Pauly, 2009

=*Nomia punctata* Westwood 1875: 213, ♂, ♀, nec Smith 1858. Type: China, BMNH (nonexamine). Syn. of Pauly (2009)

=*Nomia pilosella* Cameron 1904; 211, ♂ Holotype ♂: India, Khasia Hills, OUMNH (examined) Syn of Pauly (2009)

=*Nomia maturans* Cockerell 1912: 10. Holotype: Formose, Takao, 10. XI. 1907(not examined, sec description), Syn. of Pauly (2009)

Fig. 13

Fig. 14

Fig. 15

Fig. 16

Fig. 17

Fig. 18

Fig. 13 *Hoplonomia elliotii,* female*,* scutellum and metanotum*,* Fig. 14 *H. incerta,* female, metasoma, Fig.15 *H. callichlora*, female, metasoma, Fig.16 *H. elliotii*, female, metasoma, Fig. 17 *H. westwoodi*, female, metasoma, Fig. 18 *H. elliotii*, female, metasoma

Distribution**:** Nepal, India, China, Korea, Peninsula, Japan, Taiwan, Indonesia. In India it has been reported from Arunachal Pradesh and Meghalaya (Fig. 26)

Materials Examined: Sikkim, 27.60N; 88.45E, 1:♂leg. F.A. Noller (UZMK).

Diagnosis: Metanotum with double projection; Integumentary bands blue coloured, opalescent, band absent on the first abdominal segment; Female has the mesothorax with punctures of unequal size and the lobes of the post scutellum with obtuse process.

**4. *Hoplonomia westwoodi* Gribido, 1894**

*Nomia simillima* Smith 1875: 44, pl. II, fig. 4, ♂ = *Nomia carinata* Smith 1875: 57, ♀. Holotype ♀: Ceylon, NHMUK. **Syn. Nov.**

=*Nomia westwoodi* Gribodo 1894: 128, nom. nov. pour *Nomia simillima* SMITH 1875, nec 1863

=*Nomia erythrogaster* Cameron 1898: 61, pl. 4, fig. 10, ♂. Lectotype: India, Poona, OUMNH, designated by Baker 1993: 258

Distribution: This species is found in India, Afghanistan, Pakistan and Srilanka. In India it has been reported from Maharashtra, Andhra Pradesh,

Fig. 19

Fig. 20

Fig. 21

Fig.19 *Hoplonomia westwoodi*, female, mesosoma,
Fig. 20 *H. elliotii*, female hind leg,
Fig. 21 *H. westwoodi*, female hind leg

Karnataka, Pondicherry, Rajasthan, Tamil Nadu and West Bengal (Fig. 27)

Materials Examined: Andhra Pradesh, Hyderabad, Utrap, 17.38N 78.45E, 14-20. x.1997, 1♂, leg. Dr. Olejnicek (OOL): Bapatla, 15.93N; 80.38E, 12. xii. 2006, 1♀, coll., David, K.J.

Goa, Mormugao, 15.37N; 73.87E, vi. 1925, 1♂, ix. 1925, 1♀, leg. J.C. Bridwell (USNM).

Gujarat, Banas kantha, Deesa, 24.26N; 72.18E, iv. 1898, 1♂, x.1898, 1♂, iii. 1899, 2 males, 3 females, leg. C.G. Nurse (BMNH).

Jharkhand, Ranchi, 23.37N; 85.32E, iv. 1957, 1♂, leg. G. Angalet (USNM).

Karnataka, Mangalore, 12.86N; 74.84E xi.1926, 3♀, leg. J.C. Bridwell (USNM); Bangalore, GKVK, 930m, 12.76N; 77.74E, 1♀, 5. vi. 2013, coll. Girish; 1♀, 23. iv. 2012, coll. Arun B.C; 1♀, 7. i. 2014, 1♀, 20. i. 2014, coll. Arati Pannure; 1♂, 10. ii. 2008, coll. Nayana, E. D; 4♀ and 1♂, 4, 5, 6, ix. 2014, coll. Pradeep; GKVK, 934m, 12.76N; 77.74E, 1♀ and 3♂, 31. i. 1982, coll. B. Mallik; Hebbal, 900m, 13.03N 77.59E, 2♀, 31. x. 2014, 1♀, 11. xi. 2014, 1♀, 16. xi. 2014, 1♀, 21. ii. 2015, 1♂, 6. i. 2015, 1♂, 20. xi. 2015, coll. Zameeruddin; Sadahalli, 906m, 13.21N; 77.64E, 1♀, 6. ii. 2015, 1♀, 26. ii. 2015, coll. Zameeruddin; Belgaum, Arabhavi, 582m, 16.22N; 74.82E, 2♀, 20. ix. 2014, coll. Revansidda; Bellary, 15.14N; 76.92E 1♀, 21. ix. 2011, coll. M. Srinivasa; Chikkamagaluru, Kadur, 758m, 13.49N; 75.73E, 1♀, 24. xi. 2014, coll. Prashantha, C; Mudigere (20 km SW), 2♂, 15. iii. 2008, coll. Nayana, E; Hassan, karekere, 934m, 12.96N; 76.25E, 2♀, 23. vi. 2014, coll. Zameeruddin; Dakshina Kannada, Kankandi, 20m, 12.86N; 74.85 E, 1♀, 5. iii. 2015, coll. Prashantha, C; Kodagu, Ponnampet, 85m, 12.14N; 75.94E, 1♀, 21. vii. 2015, coll. Prashantha, C; Kolar, Horticulture college, 830m, 13.13N; 78.16E, 1♀, 16. xii. 2014, coll. Zameeruddin; Koppal, Munirabad, 466m, 15.27N; 76.32E, 1♀, 5. xii. 2012, coll. Najeer; Mandya (Sasalu), 841m, 12.52N; 76.89E, 2♀, 6. x. 2014, coll. Pradeep; VC farm, 727m, 12.52N; 76.89E, 2♂, 10. viii. 1982, coll. B. Mallik; Mysore, Chinnamballi, 716m, 12.09N; 76.83E, 2 ♀, 19. vii. 2015, coll. Prashantha, C; COH, 824m, 13.13N; 78.16E, 1♂, 20. vii. 2015, coll. Prashantha, C; Hunsur, 12.30N; 76.29E, 1♀, 18. iv. 2009, Dhanyavathi, P.N; Nanjangud, 24.64N; 72.77E 1♂, 24. i. 2009, coll. Dhanyavathi, P. N; Banur, 13.32N; 75.77E 1♀, 23. iv. 2009, Dhanyavathi, P.N. Udupi, Brahmavar, 13.43N; 74.74E, 1♂, 12. iv. 1985, coll. A.R.V Kumar.

Kerala, Malappuram, Calicut University, botanical garden, 11.13N; 75.89E 27. ix. 1986, 1♂, 1♀, leg. E.E. Grissell (USNM); Palakkad, Walayar Forest, 10.84N; 76.84E 700ft, ix.1959, 1♀x. 1959, 5♂ 9♀, leg. P.S. Nathan (RMNH), ix. 1953, 1♀ leg. P.S. Nathan (IRSNB).

Fig. 22. *Crocisaspidia buddha*, female

Fig. 23

Fig. 22 *Crocisaspidia buddha* female; Fig. 23 *C. buddha*, male

Maharashtra, Amraoti Dist., Melghat Tiger res, Dhakna, 21.15N; 77.64E, 1300ft, 21. ii. 1976, 1♂, leg. M.L. Ripley (USNM); Raigad, Matheran, 18.98N; 73.26E iii. 1899, 1♀ (BMNH), 3♂ 3♀ iv. 1899, 1♂, leg. C.G. Nurse (BMNH); Bombay Presidency, 19.04N; 72.90E, 1♀ (BMNH).

Orissa, Teypone, 20.5N; 84.4E, 1775ft, ix. 1958, 5♀ x. 1958, 6♀ leg. P.S. Nathan (RMNH).

Pondicherry, Karaikal, 11.92N; 79.83E, 22. ii. 1946, 1♂ leg. P.S. Nathan (USNM); i. 1964, 1♂, iii. 1964, 1♂, vii. 1964, ♀ leg. P.S. Nathan (SMUK); xii. 1958, 1♀, .1959, 2♂, iii. 1962, 2♂, 1♀ iii. 1962, 25♂, iv. 1962, 58♂, 2♀ vi. 1962, Ashmead, 5♀ vii. 1962, 2♂, 4 ♀, viii. 1962, 3♀, iii. 1964, 1♀ leg. P.S. Nathan (RMNH); Nettapackam, 11.86N; 79.63E, x. 1963, 1♂, leg. P.S. Nathan (SMUK); 10km N. Auroville, Discipline Farm, 12.00N; 79.80E, 2. iii. 2010, 1♀, leg. & col. F. Burger: Karaikal Territory, Kurumbagaran, 10.92N; 79.83E, 12. viii. 1954, 1♀, ix. 1954, 1♀, leg. P.S. Nathan (IRSNB).

Rajasthan, Abu, 24.64N; 72.77E 1♀, leg. C.G. Nurse (NHMUK).

Sikkim, 27.60N; 88.45E 1♂, leg. Fedschlotter (UZMK).

Tamilnadu, Coimbatore, 11.00N; 76.96E 1400ft, vi.1965, 1♂, 2 ♀, ix. 1964, 1♀, leg. P. S. Nathan (SMUK), 1. vii. 1953, 2♀, ix. 1953, 2♀, xi. 1953, 3♂ leg. P.S. Nathan (IRSNB); Palani Hills, 10.49N; 77.50E Educi Mtn, 19. xi. 2005, *Mentha* sp., 1♂, leg. *et* col. F. Burger, ix. 1951, 1♂, leg. P.S. Nathan (USNM); Tuticorin, 8.80N; 78.14E, 18. x.1938, 1♀

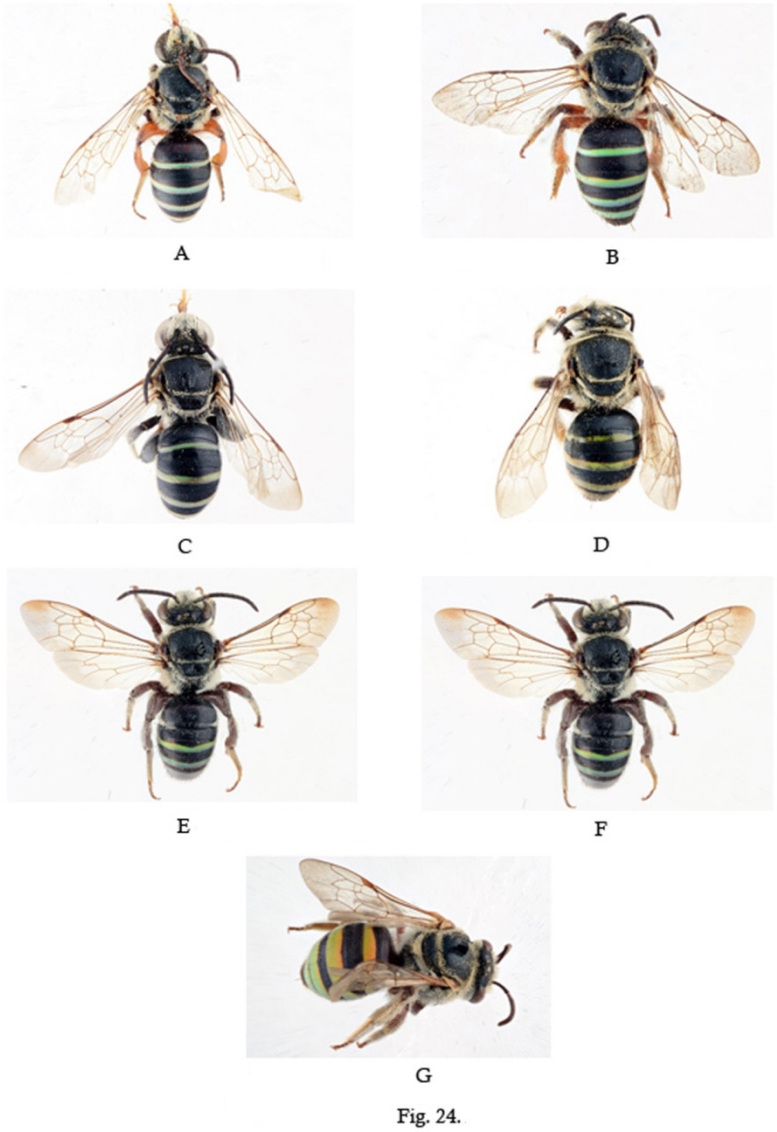


Fig. 24 A- *Hoplonomia westwoodi* male, B- *H. westwoodi* female, C- *Hoplonomia elliotii* male, D- *H. elliotii* female, E.- *H. incerta* male, F- *H. incerta* female, G- *H. callichlora* female

(BMNH), vii. 1958, 1♂, viii. 1958, 1♀, ix.1959, 1♂, iv. 1962, 12 ♀, leg. P.S. Nathan (RMNH); Annamalai Hills, 11.11N; 77.35E, Cinchona, 3500ft, v.1964, 1♂, leg. P.S. Nathan (RMNH); 1.vii.1953, 2♀ Tranquebar, 11.02N; 79.85E, vi. 1951, 6♀ 9. xii.1951, 1♂, vii.1953, 3♀ xii. 1953, 4♀, leg. P.S. Nathan (IRSNB); Nilgiris Hills, Moyar camp, 11.41N;76.69E, 2100ft, iv.1954, 13♂, leg. P.S. Nathan (IRSNB); Nilgiris hills, Singara, 11.34N; 76.81E, 3400ft, v. 1954, 1♀, 1♂, leg. P.S. Nathan (IRSNB).

Diagnosis: Similar to *H. elliotii* with posterior lateral angles of the scutellum tuberculate and post scutellum with two teeth; but smaller; posterior femur and tibia not so swollen and thick; inner angle of the apex of tibia is produced and rounded and not forming flat truncate process as in *H. elliotii;* abdomen beneath and posterior legs pale rufotestaceous

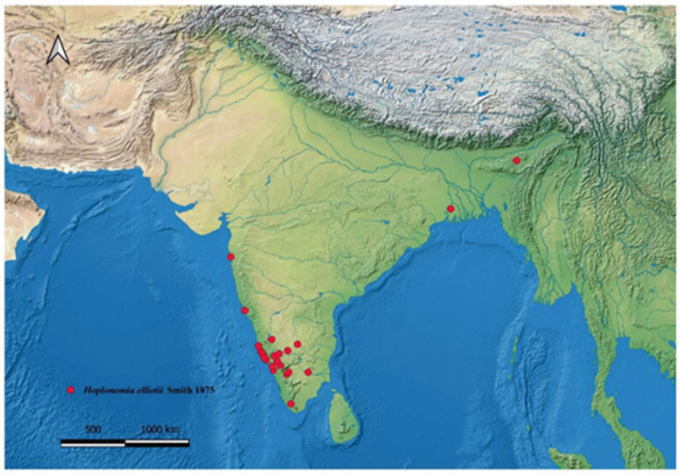


Fig: 25

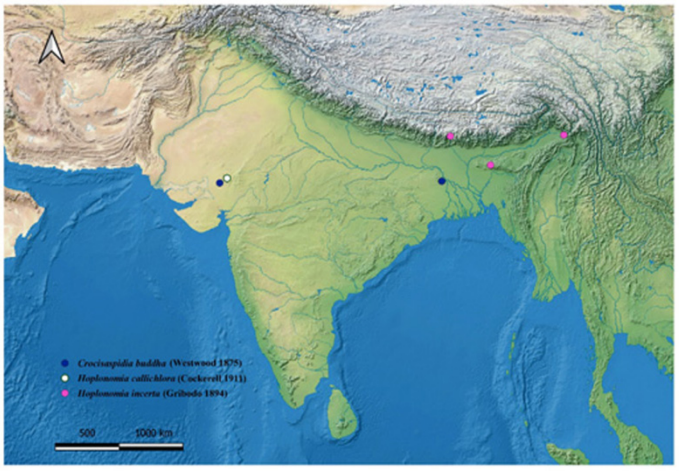


Fig:26

Fig. 25 Distributional map of *Hoplonomia elliotii* recorded so far from India; Fig. 26 Distributional map of *Crocisaspidia buddha*, *H. callichlora*, *H. incerta* recorded so far from India

**Subfamily Nomiinae,**
**Genus: *Crocisaspidia* Ashmead**

Diagnosis: Scutellum is characteristic as compared to other Nomiinae for its lateral projections are flat with raised carinate edges and extend straight back not upward from the rest of scutellum. All species are larger with 9 -16mm length. Enamel bands on tergites are on apical margins. In females basal plate of hind tibia is completely rounded. The propodeal area 'V' shaped ending on the posterior face vertically, horizontal only in its lateral parts. Tegulae is in the form of ears.

Distribution**:** This genus is basically Afrotropical (9 species), one species in Madagascar, and three in the Arabian Peninsula. Currently only a single species is known from India and it is known from West Bengal and Gujarat.

**5. *Crocisaspidia buddha* (Westwood, 1875)**

*Nomia budha* Westwood, 1875: 209, pl. IV, fig. 1, ♂. Lectotype ♂: India, OUMNH, designated by Pauly 1990: 48 (reviewed)

= *Nomia bahadur* Nurse, 1904: 568, ♀, ♂.

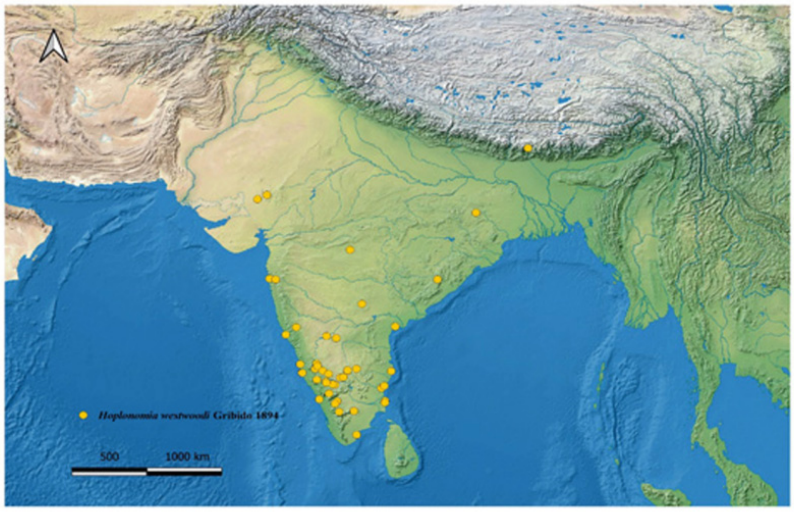


Fig:27

Fig. 27 Distributional map of *Hoplonomia westwoodi* recorded so far from India

Lectotype ♀: Deesa, BMNH (examinéd) Distribution: West Bengal, Gujarat (Fig. 26)

Materials Examined: West Bengal, 23.55N 88.57 Chapra (Mackenzie), 1♂ (NHMUK).

Gujarat, Deesa, 24.25N; 72.18E ix.1901, 1♀ leg. C.G. Nurse (NHMUK)

Diagnosis: Males are with characteristically shaped posterior tibias. In females integumentary bands are pale blue green in color; all bristles of the tibia and posterior tarsi with whitish grey bristles; first tergite with sparse punctuations.

**Key to Genera and Species of *Hoplonomia* and *Crocisaspidia* of India**

Males: antenna of 13 segments; hind leg without scopal hair and femora often thickened; no sting.

Females: antenna of 12 segments; hind legs with scopal hairs; sting.

**Males** (male of *H. callichlora* unknown)

1. Scutellum with a pair of lateral ear-shaped lamella, metanotum with a pair of large projections (Fig.1) basitarsus of hind leg is large .....***Crocisaspidia buddha***

- Scutellum mutic (Fig. 2) or with a pair of much reduced teeth (Fig. 3), metanotum with a pair of narrow projections (Figs. 2, 3); basitarsus of hind leg narrow..... ***Hoplonomia***

2. First tergum without enamel-like band, but with lateral tuft of white hairs; terga 2 to 4 with yellow, green or blue enamel-like apical bands (Fig.7).....***H. incerta***

- First to fourth terga with yellow, green or blue enamel-like apical bands (Figs. 8, 3)

3. Hind leg nearly completely black, only the apical lobe of the tibia amber, hind femur very thick (Fig.10).....***H. elliotii***

- Hind leg nearly completely amber, hind femora moderately thick (Fig.11).....***H. westwoodi***

Females

1. Scutellum with lateral ear-shaped lamella, metanotum with a pair of large projections (Fig.12) .....***Crocisaspidia buddha***

- Scutellum mutic, metanotum with a pair of small and narrow projections (Fig.13) .....Genus ***Hoplonomia***

2. First tergum without enamel-like band, but with lateral tuft of white hairs; terga 2 to 4 with yellow, green or blue enamel-like apical bands (Fig.14).....***H. incerta***

- First to fourth terga with yellow, green or blue enamel-like apical bands.....3

3. First tergum with enamel-like apical band about twice as broad, covering the totality of the apical depression (Fig.15).....***H. callichlora***

- First tergum with enamel-like band not exceeding half of the apical depression.....4

4. Scutum with dense punctuation (Fig.18); hind legs largely amber (Fig. 20).....***H. elliotii***

-Scutum with more spacer punctuation (Fig. 19); hind legs largely black (Fig. 11).....***H. westwoodi***

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# Taxonomical studies of dragonfly nymphs (Odonata, Libellulidae) using their exuviae

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**ABSTRACT:** Taxonomic studies of dragonfly nymphs were done up to species level using their exuviae. Exuviae, being the last instar larval skin, possess all larval features. These features can be used for the identification of odonate nymphs up to species level. Seven species belonging to the family Libellulidae were identified using the features present on exuviae. This is a non-invasive method that can be used for the taxonomic studies of dragonfly nymphs without rearing them in the laboratory. The study describes the morphological features of seven species of dragonfly nymphs belonging to family Libellulidae using exuviae. A taxonomic key for the identified exuviae were also provided.


**KEYWORDS:** Species identification, non-invasive method, taxonomic key

## INTRODUCTION

Odonata comprises dragonflies and damselflies, one of the most ancient insect groups. In Kerala, a total of 174 species of odonates have been reported of which 65 are endemic to the Western Ghats, and 10 to India (Gopalan *et al*., 2022). The kole wetlands of central Kerala reported 44 species of odonates (Chandran *et al*., 2021). Being amphibiotic and hemi-metabolous insects, adults are terrestrial, and nymphs have aquatic modes of life. Adult females, after mating, lay their eggs in different aquatic ecosystems. Egg hatch into nymphs. Dragonfly nymphs are strictly predacious, and by using their extensible labium, they capture aquatic insects, crustaceans, small molluscans, larvae of other insects, and oligochaetes. At the end of the aquatic life, the nymph finds a support, such as a rock or plant stem, where it can tear up its skin and enter the aerial life as an adult. The skin that remains on the rock or plant stem is called exuviae, and they are nothing more than the dried skin of the last larval instar and are therefore vulnerable. Since the exuvia is the moulted skin of the penultimate instar, it possesses all the larval features (Adambukulam and Kakkassery, 2013a, b).The main features that can be used for the taxonomic identification larva includes shape of the labium, number and shape of antennal segments, number of premental and palpal setae, presence or absence of mid dorsal spines, and position of lateral abdominal spines, shape of caudal appendages etc. These features show variation within the family, genus and species level and hence can be used to study the larval taxonmy. Taxonomical studies of dragonfly nymphs were usually conducted either by collecting nymphs from the field or eggs from an ovipositing female in the field and rearing them

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in the laboratory. In Kerala, Nirmalakumari and Balakrishnan (1981, 1983) studied the life history of the nymphs of *Rhodothemis rufa* (Rambur, 1842) and *Urothemis signata* (Rambur, 1842) by rearing the larvae in the laboratory. No further studies on Odonate nymphs were reported from Kerala till 2013. Adambukulam and Kakkassery (2013a, b) conducted the larval description studies of dragonfly nymphs by using their exuviae. A typical libellulidae larvae can be identified by their spoon shaped labium and well-developed premental and palpal setae. Interspecific variations were seen in the number of setae. The presence or absence of mid dorsal abdominal and lateral abdominal spines can also be taken as a taxonomic feature. The present study describes the morphological features of seven species of dragonfly nymphs belonging to family Libellulidae using their exuviae.

## MATERIALS AND METHODS

During the exuvial survey in Thrissur district, exuvia were collected from a pond (10°27'50"N; 76°11'31"E) located in a paddy field and also from a man-made cement tank located in Ammadam, a small village, 8 km away from Thrissur. Collections were done during the early morning hours, because the process starts in late midnight to avoid predation pressure and hence the collector had the opportunity to see the adult just emerging from the exuviae, which helped to identify the species at the primary level in the field itself. The specimens (exuviae) were collected in plastic bottles of the photographic film and brought into the laboratory. Wet specimens were dried by placing them under an incandescent lamp and then they were pinned and preserved as dry specimens for analysis. The exuviae were dissected by observing through the Stereo Dissection Microscope (CZM 4, LABOMED) for analyzing the larval features present on exuviae. The description of the general morphology follows Tennessen (2019). The SEM images of the main features present on the exuviae (Figs. 1 A-F) were taken using JEOL 6390LA. Exuvia sample was smeared over the multisided adhesive carbon tape fixed on specimen stubs and over-coated with gold using JFC 1600. This ion-sputtering device performs rapid and efficient gold coating on the microscopic specimen, allowing surface visualization. The SEM measurements were performed at 15 kV accelerating voltage .Different magnifications were used, as indicated on the images. The main taxonomic features present on exuviae includes shape of the labium, the number of premental and palpal setae, setaceous margin of palpus, crenations on palpus, presence or absence of mid dorsal protuberances, their shape (spine like or hook like), lateral spines, extension of wing pads, presence of hairs at base of the abdomen and length and size of caudal appendages. The features observed on exuviae were compared with original larval descriptions by Kumar (1984, 1988, 1990) and Hussain and Riaz (1999a, b). The final plates were prepared using Leica S80AP0 stereo microscope (Leica Microsystems, Germany) with an in-built camera (Leica MC 170 HD) and LAS auto-imaging software. The specimens were retained in the Entomology museum of Department of Zoology, St. Thomas College Thrissur.

## RESULTS AND DISCUSSION

A total of 105 exuviae were collected during the study. They include *Acisoma panorpoides* Rambur, 1842 (12 nos.), *Bradinopyga geminata* (Rambur, 1842) (22 nos.), *Neurothemis tulia* (Drury, 1773) (9 nos.), *Orthetrum sabina* (Drury, 1773) (26 nos.), *Rhyothemis variegata* (Linnaeus, 1763) (12 nos.), *Tholymis tillarga* (Fabricius, 1798) (11 nos.) and *Zyxomma petiolatum* Rambur, 1842 (13 nos.). All belong to the family Libellulidae.

### 1. *Acisoma panorpoides* (Figs. 2 A-F)

A small exuvia having dark brown colour; body length ranges between 16.2–18mm; head small, triangular with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of mid leg at rest; distal margin of prementum is triangular, and projecting; premental setae11+11; palpal setae 8+8; distal margin of palpus formed into crenations with claviform setae; thorax bears long legs; hind leg extends beyond the body; wing pads diverging; hind wing pad extends up to the middle of the 7$^{th}$ segment; abdomen is more or less oval shaped; mid dorsal protuberances absent;

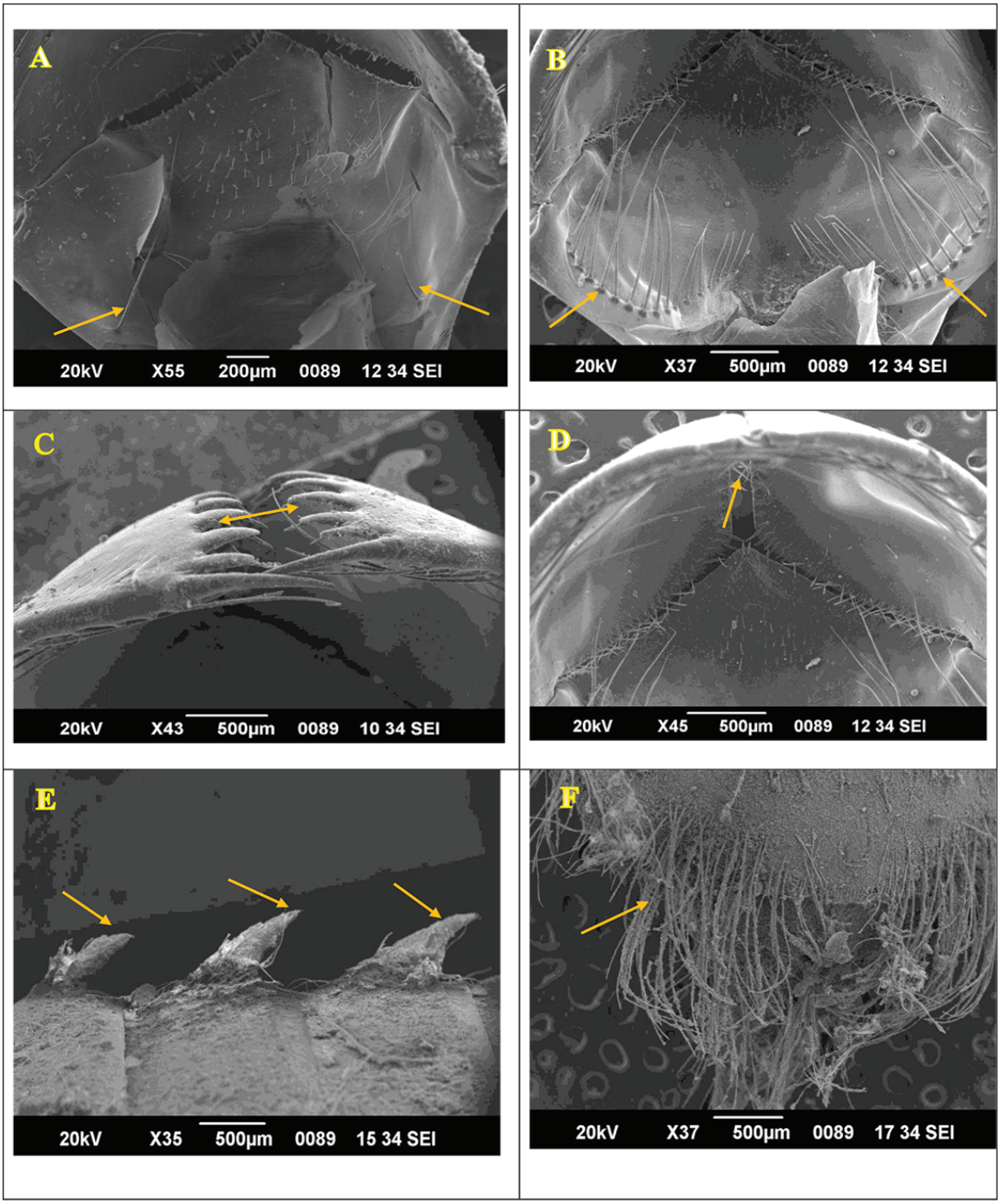


Fig. 1 Taxonomic features on Exuviae. A-Premental setae in *Rhyothemis variegata*; B-Premental setae in *Bradinopyga geminata*; C- Palpal crenations in *Pantala flavescens*; D- Palpal spines in *B. geminata* E- Mid dorsal protuberances in *Orthetrum sabina*; F- Abdominal hairs in *O. sabina*

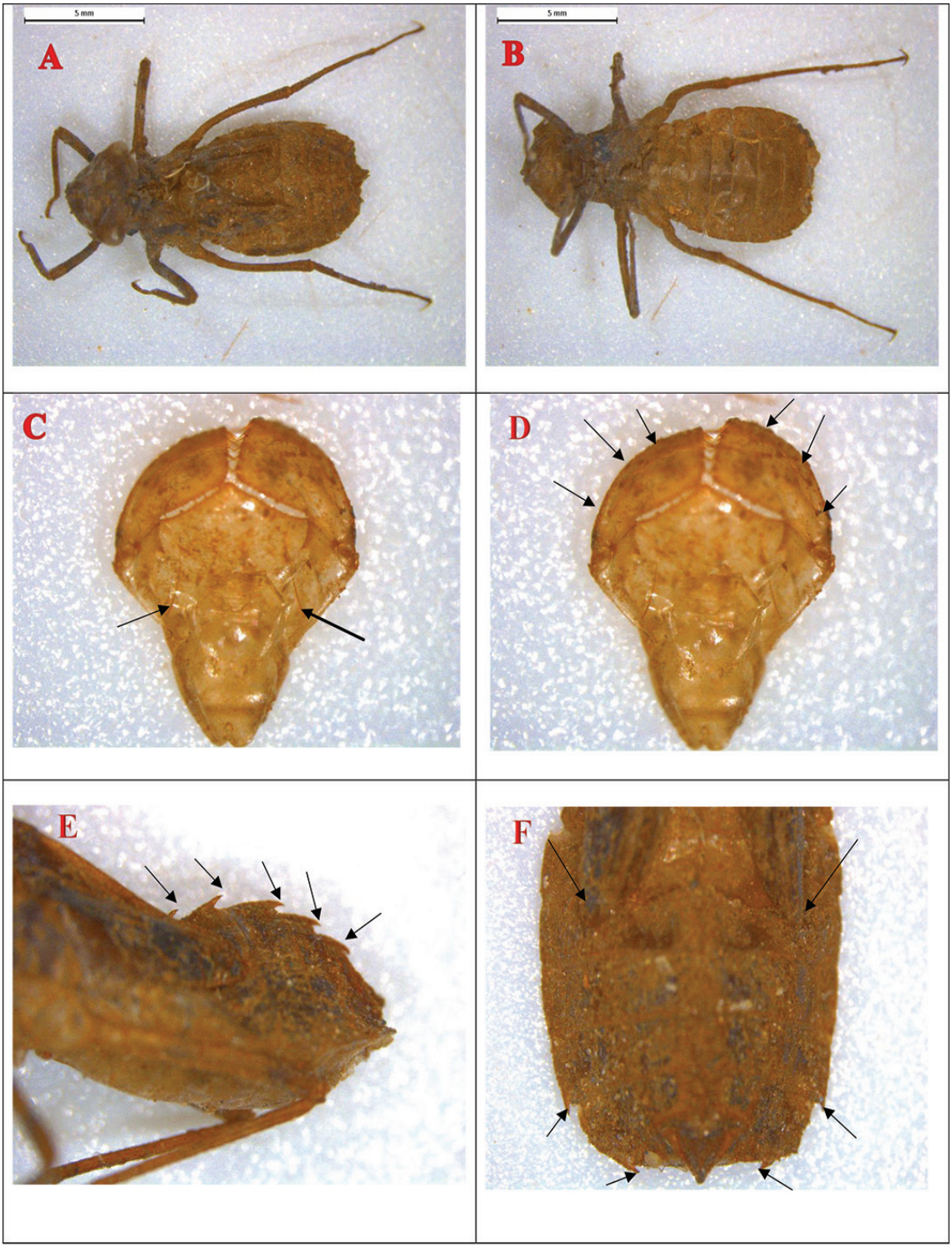


Fig. 2 *Rhyothemis variegata.* A-Dorsal view; B-Ventral view ; C-Premental setae D-Palpal setae; E- Mid dorsal spines F- Lateral spines and Wing pads

lateral spines on S8 and S9; S10 smaller than anal appendages; epiproct triangular, broad at the base; cerci shorter than epiproct and are pointed; paraproct equal length of epiproct. These features were compared with the larval features given (Kumar, 1984) by rearing the larvae in the laboratory.

Remarks: *A. panorpoides* is widely distributed in India, Nepal, China, Sri Lanka, Burma, Thailand, Malaysia, Indonesia, Japan, Taiwan, Philippines, Africa and Madagascar. In Kerala they usually associated with ponds and canals in paddy fields and also near to other stagnant water bodies and their oviposition occurs just after first monsoon rain. The emergence occurs at the end of monsoon season. The exuviae found to be attached to lower side of aquatic plants at a height of 10-20cm above water level.

**2. *Bradinopyga geminata* (Figs. 3A-F)**

A medium sized transparent exuvia; body length ranges between 19–22mm; head small, triangular with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae19+19;palpal setae 16+16; distal margin of palpus crenated with claviform setae; long slender legs with dark bands on femur and tibia ; wing pads diverging; hind wing pad extends up to the middle of the 6$^{th}$ segment; abdomen is broader than head; dorsally convex; ventrally flattened; irregular dark spots present; abdominal segments S2–S8 almost equal sized; S9–S10 tapering; mid dorsal protuberances absent; well-developed lateral spines on S8 andS9; S10 smaller than anal appendages; epiproct triangular, acuminate broad at the base; cerci shorter than epiproct and acuminate; paraproct equal length of epiproct. The exuvial features were compared with the larval features Sangal and Kumar (1970).

Remarks: This species is widely distributed throughout the oriental region. They usually breed in overhead tanks and garden ponds. The exuviae were usually found attached to manmade garden ponds. But in garden ponds in which ornamental fishes are growing their emergence is found to be less. Sometimes they emerge in large numbers. It is a multivlotine species and emerges throughout the year.

**3. *Neurothemis tulia* (Figs. 4 A-F)**

A medium sized exuvia with brown colour; body length ranges between 11–14mm; head small, pentagonal in outline; with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae13+13;palpal setae 8+8; distal margin of palpus crenated; long slender legs with dark bands on femur and tibia; wing pads diverging; hind wing pad extends up to the middle of the 5$^{th}$ segment; abdomen is broader than head; dorsally convex; ventrally flattened; more or less oval shaped; mid dorsal protuberances absent; lateral spines on S8 and S9; S10 smaller than anal appendages; epiproct triangular, acuminate broad at the base; cerci shorter than epiproct and acuminate; paraproct equal length of epiproct. The features were compared with studies of Kumar (1988) and Begum *et al.* (1990).

Remarks**:** *N. tulia* is an extremely widespread common species, found throughout mainland tropical and subtropical Asia. In Kerala the species is distributed everywhere. They are also multivoltine and emergence occurs all the year around. They breed in small ponds, streams and rivers etc. A variation in the number of premental and palpal setae is observed in Indian species where it is 13 and 8 respectively (Kumar, 1988), for the Bangladesh specimen it is 10 and 9 from Bangladesh (Begum *et al.,* 1990) The difference in the number may be due to the geographical intra specific variations. The collected exuviae show features of Indian representative.

**4. *Orthetrum sabina* (Figs. 5 A-F)**

A medium sized exuvia with muddy brown colour; body length ranges between 19–22mm; head small, pentagonal in outline; with compound eyes projecting antero-laterally; antennae seven

segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 7+7;palpal setae 7+7; distal margin of palpus crented; legs robust and covered with hairs; wing pads diverging; hind wing pad extends up to the middle of the 6th segment; abdomen is broader than head; dorso-ventrally flattened; more or less oval shaped; spine like mid dorsal protuberances present from S4–S9; setae along posterior margin of all abdominal terga; lateral spines absent; sternum of S9 bears a bunch of hairs; S10 smaller than anal appendages; anal appendages are hairy; epiproct triangular, acuminate broad at the base shorter than paraprocts; cerci less than half length of epiproct and acuminate; paraproct acuminate equal length of epiproct.

Remarks: *O.sabina* is widely distributed in Ethiopian, Oriental and Australian region. Kumar (1973) studied the life history of *O. sabina*, and in his studies that the final instar has 11+11 premental setae and 7+7 palpal setae respectively. But the exuvia collected by the authors show variation in the number of premental and palpal seate 7+7 premental and 7+7 palpal setae and this may be due to geographical variation. In Kerala it is multivoltine species and emerges in large number during pre and post monsoon seasons.

**4. *Rhyothemis variegata (*Figs. 6 A-F)**

A small sized exuvia with grayish black colour; body length ranges between 11–13mm; head small, pentagonal in outline; with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 1+1; palpal setae 5+5; distal margin of palpus crenated; legs long and slender ; hind leg extending beyond the abdomen; wing pads diverging; hind wing pad extends up to the middle of the 5th segment; abdomen is broader than head; dorso-ventrally flattened; oval shaped; spine like mid dorsal protuberances present from S3–S9; lateral spines present on S8–9; S10 smaller than anal appendages; anal appendages are hairy; epiproct triangular, acuminate broad at the base shorter than paraprocts; cerci less than half length of epiproct and acuminate; paraproct acuminate equal length of epiproct.

Remarks: This species is distributed throughout the Oriental region. The larvae have been described by Chowdhurry and Akhteruzzaman (1981). In their description the larvae possess 2+2 premental setae but in the collected exuviae it is 1+1. In Kerala it usually emerge during post monsoon season in large numbers.

**6. *Tholymis tillarga* (Figs. 7A-F)**

A small sized exuvia with grayish black colour; body length ranges between 11–13mm; head small, pentagonal in outline; with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 7+7; palpal setae 5+5; distal margin of palpus crenated; long, slender legs with dark bands; hind leg extending beyond the abdomen; wing pads diverging; hind wing pad extends up to the middle of the 5th segment; abdomen is broader than head; dorso-ventrally biconvex; tapering towards the distal end; hook like mid dorsal protuberances present from S3–S9; lateral spines present on S8–9; S10 smaller than anal appendages; anal appendages are hairy; epiproct triangular, acuminate broad at the base; cerci shorter than epiproct and acuminate; paraproct acuminate equal length of epiproct.

Remarks: This species has a wide distribution in all parts of the world except Europe and the Americas. The larvae are sluggish and bottom dwellers and were described (Kumar, 1973; Chowdhury and Akhteruzaman, 1981). In Kerala the emergence occurs during post monsoon season in large numbers.

**7. *Zyxomma petiolatum* (Figs. 8 A-F)**

A medium sized exuvia with blackish grey colour; body length ranges between 17–20mm; head small, pentagonal in outline; with compound eyes

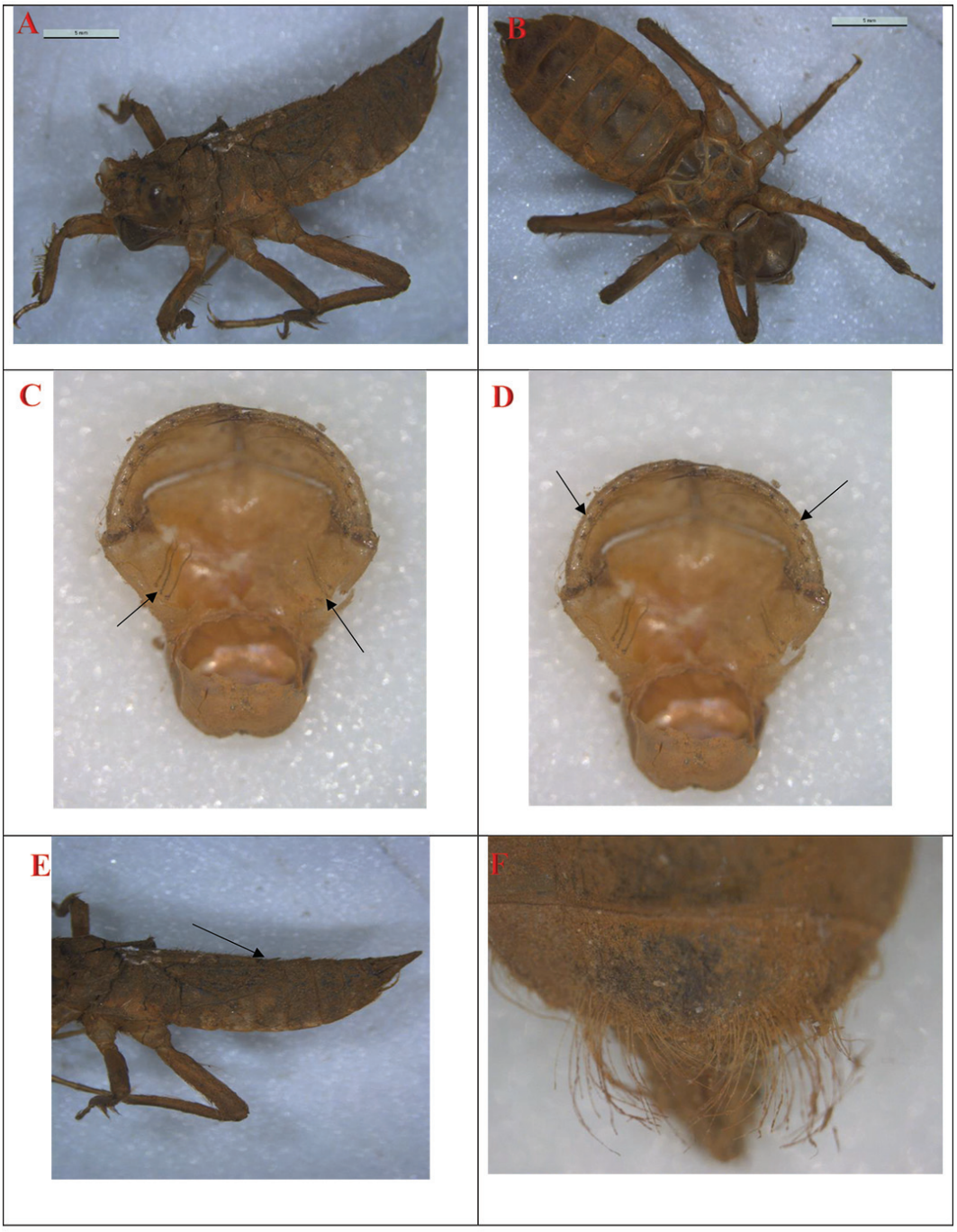


Fig.3 *Orthetrum sabina.* A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Mid dorsal spines F- Hairs on the abdomen

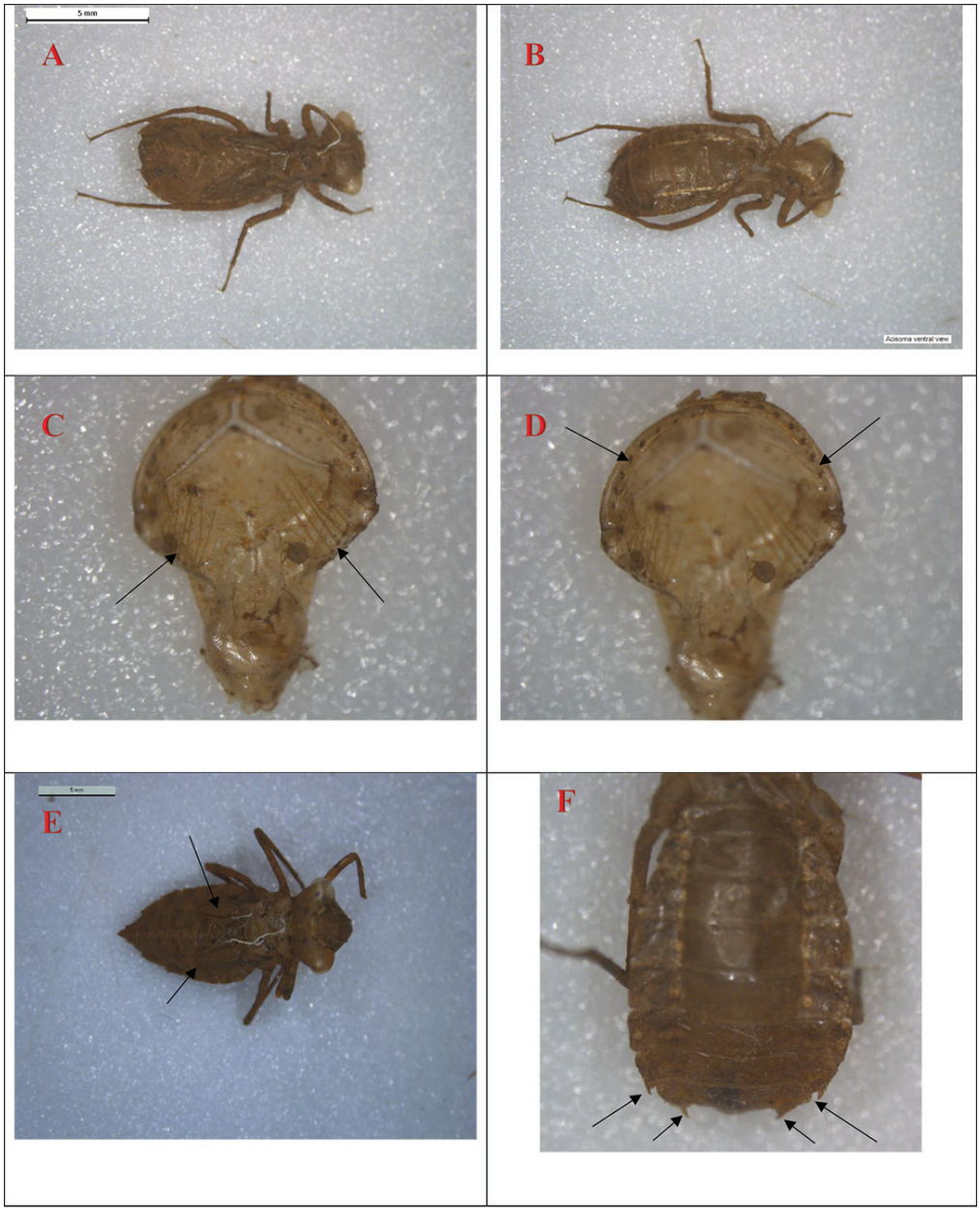


Fig. 4 *Acisoma panorpoides.* A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Wing pads; F- Lateral spines on the abdomen

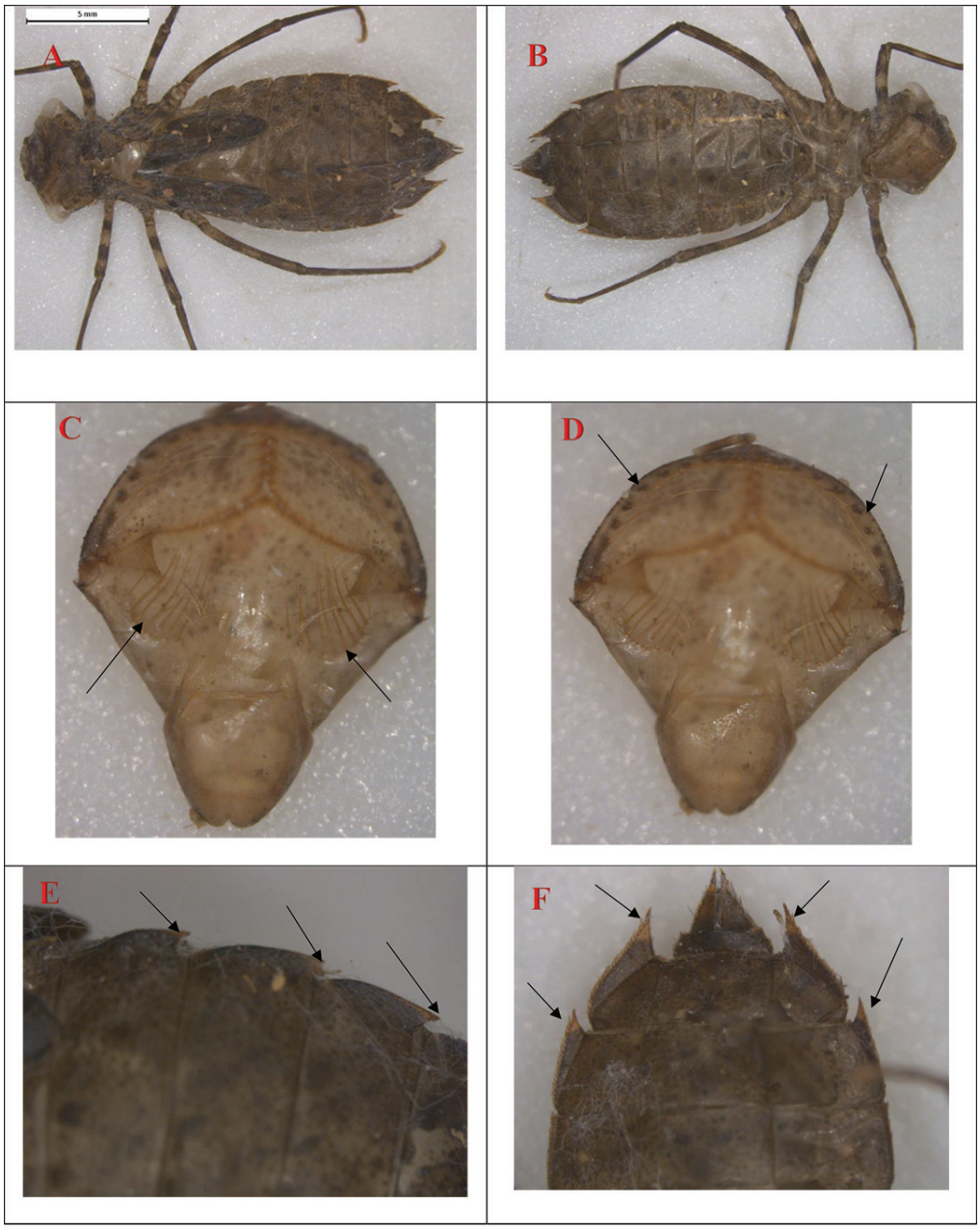


Fig 5: *Zyxomma petiolatum.* A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Mid dorsal protuberances; F- Lateral spines on the abdomen

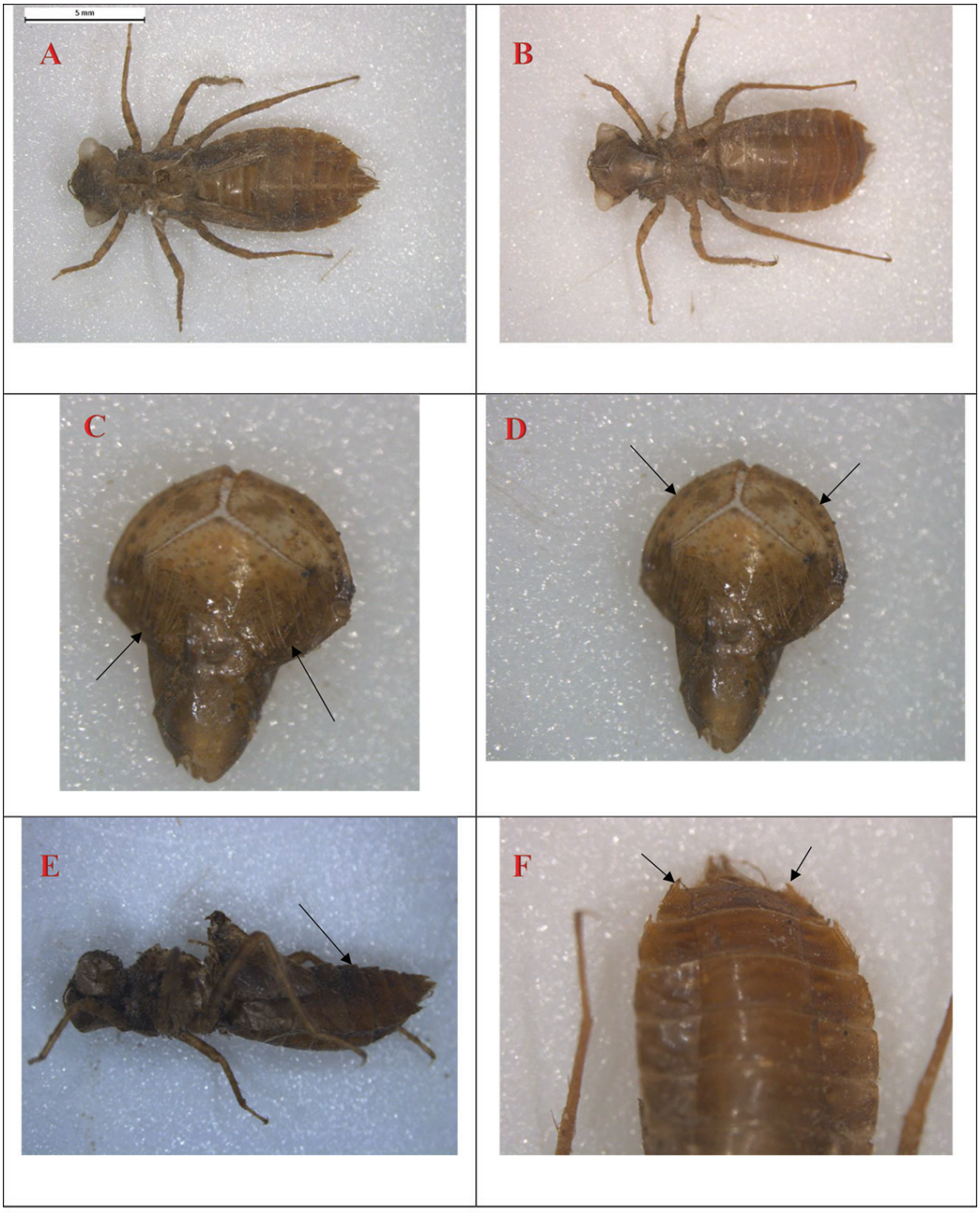


Fig. 6 *Neurothemis tulia.* A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Mid dorsal protuberances; F- Lateral spines on the abdomen

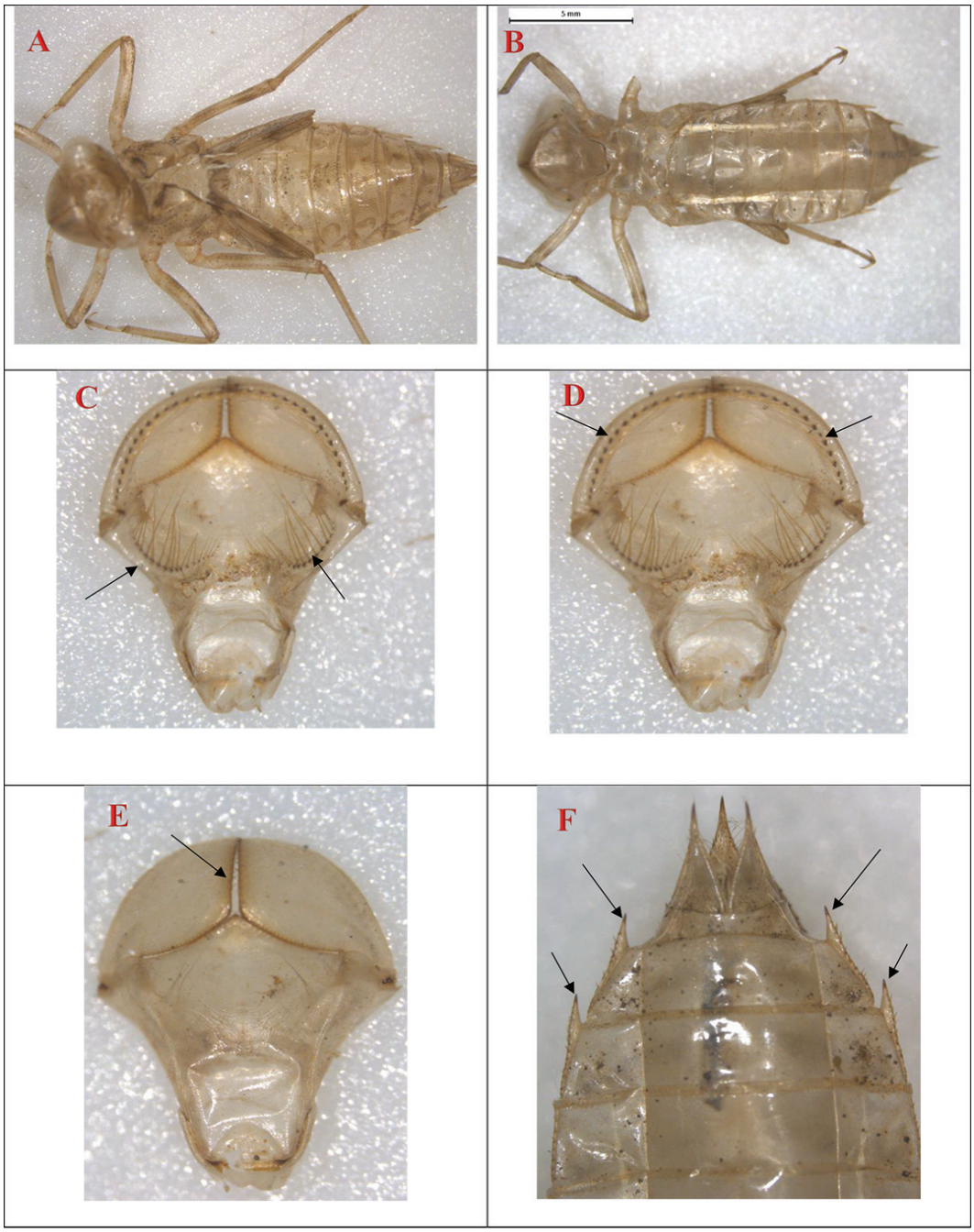


Fig. 7 *Bradinopyga geminata.* A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Palpus with crenations; F- Lateral spines on the abdomen

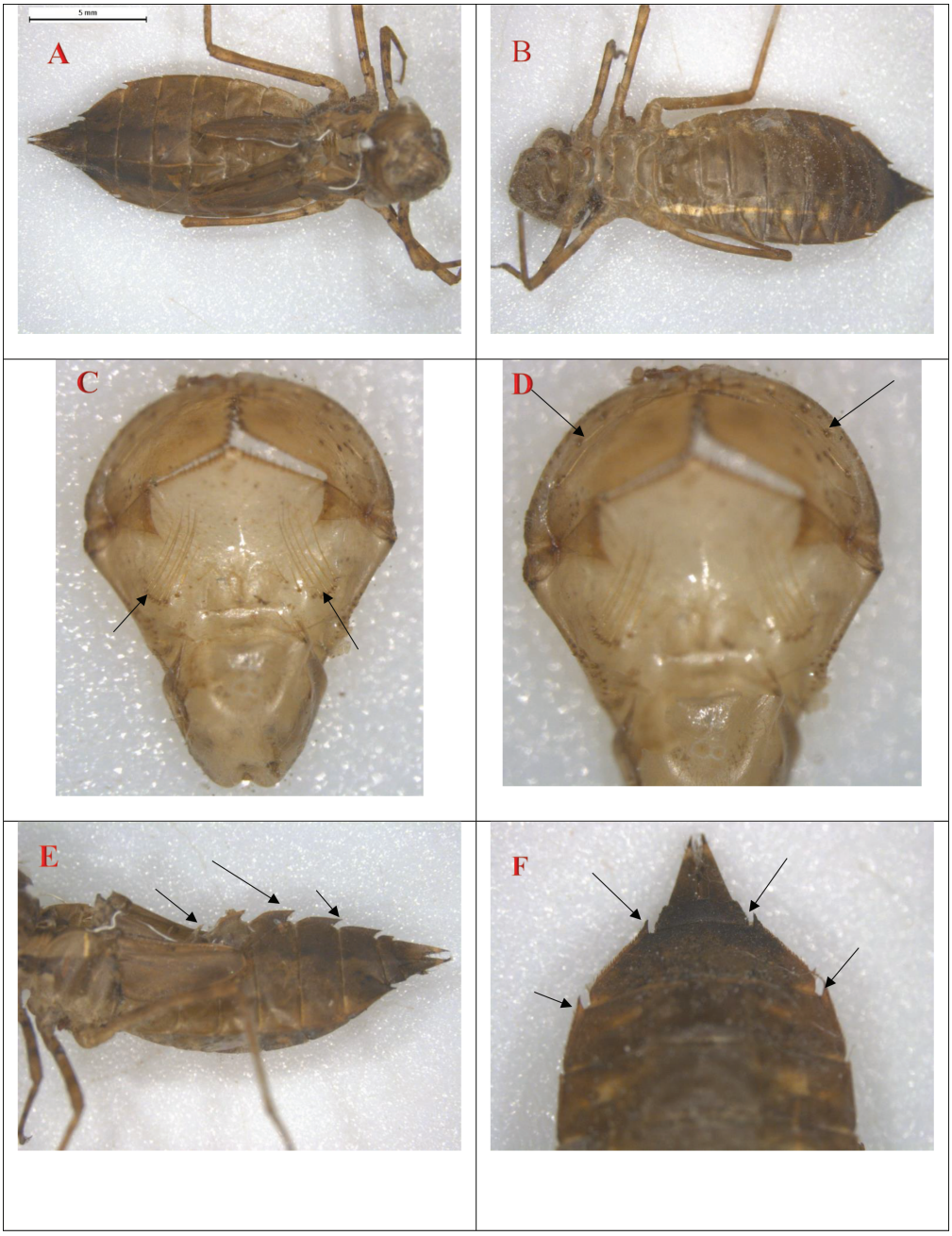


Fig. 8 *Tholymis tillarga.* A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Mid dorsal protuberances; F- Lateral spines on the abdomen

projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 12+12;palpal setae 8+8; distal margin of palpus crenated; legs long and slender ; hind leg extending beyond the abdomen; wing pads diverging; hind wing pad extends up to the middle of the 5th segment; abdomen is broader than head; dorso-ventrally biconvex; tapering towards the distal end; hook likemid-dorsal spines present from S1–S10, spines on S1–S3 and S10 were very small,S4–S9 prominent, sharp and pointed; postero lateral spines on S8–9, spine on S9 is longest and reaching up to the length of epiproct; S10 smaller than anal appendages; anal appendages are hairy; epiproct triangular, acuminate broad at the base; cerci shorter than epiproct and acuminate; paraproct acuminate equal length of epiproct.

Remarks: It is a common species in India, Myanmar and Sri Lanka and is a crepuscular dragonfly appearing shortly before dusk and coming often to house lights. Breed in small stagnant pools and even in domestic wells. The larvae was studied by (Kumar, 1973: Chowdhury and Akhteruzzaman, 1981).The breed in natural water boides or man-made water bodies.They are multivoltine species and emerge in all seasons throughout the year.

**Key to the 7 species of Libellulidae**

1. Mid dorsal abdominal spines absent.....2

Mid dorsal abdominal spines present.....5

2. Body length <15mm; palpal setae<10.....3

Body length >15mm; palpal setae >10.....4

3. Wing pads extending up to middle of S5; premental setae 13+13; palpal setae 8+8; lateral spines on S8–9;.....***Neurothemis tulia***

Premental setae 11+11; palpal setae 7+7; lateral spines on S9–10; wing pads extending up to middle of S7.....***Acisoma panorpoides***

4. Wing pads extending to S6; premental setae 17+17; palpal setae 15+15; lateral spines on S8–9; .....***Bradinopyga geminata***

5. Abdominal lateral spines absent on S8–9.....6

Abdominal lateral spines present on S8–9.....7

6.Premental setae 7+7;palpal setae 7+7;long tapering abdomen with tuft of hairs at S9.....***Orthetrum sabina***

7. Abdomen with dark mid dorsal stripe from S3–9.....8

Abdomen without mid dorsal stripe form S3–9...9

8. Premental setae 7+7; palpal setae 5+5; mid dorsal protuberances from S4 9.....***Tholymis tillarga***

9. Mid dorsal protuberance from S3–9 are spine like; premental setae 1+1; palpal seate5+5; .....***Ryothemis variegata***

Mid dorsal protuberances from S1-10 are hook like;premental setae 12+12; palpal setae8+8; .....***Zyxomma petiolatum***

This present work was focused on morphological features of exuvia (final instar exoskeleton) for identifying dragonfly nymphs up to species level. Most of the nymphal studies of dragonflies were based on the rearing of larvae that directly collected from the field or by collecting the eggs from ovipositing females from the field. Both methods are time consuming Thus studies, using exuviae can be considered as a noninvasive method for nymphal diversity. This methodology of exuvia collection from the field is suggested for the those species which are difficult to observe as adult and also for identifying larval features endemic and endangered dragon fly species. The presence of exuviae also indicates the successful breeding sites of dragonflies and thus health of the aquatic ecosystem can be assessed. The counts of exuviae may give a clear picture of real dragonfly abundance and diversity than do male biased counts, and that numbers emerging from a small or medium sized water body can usually be monitored by a single researcher (Corbet and Hoess, 1998).

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# Screening of wild *Ipomoea* genotypes for resistance against sweet potato weevil *Cylas formicarius* F. based on multiple choice bioassay and phytochemical constituents

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**ABSTRACT:** Screening of wild *Ipomoea* spp. and identification of new sources of resistance to the sweet potato weevil (*Cylas formicarius* Fabricius) with *I. palmata, I. mauritiana, I. obscura, I. triloba* were carried out. The leaves, vines and tubers of the different *Ipomoea* sp. were screened using multiple choice bioassay. The insect feeding holes on *I. mauritiana* leaves (1.67+1.528), vines (7.67±2.96) and tubers (12.67+2.309) was significantly less compared with other *Ipomoea* sp. Further, the two-choice bioassay was done, using *I. batatas* and *I. mauritiana* for comparison. Based on the morphological screening different phytochemical constituents was identified using GC-MS analysis of the methanolic extract of roots of selected *Ipomoea* spp. (*I. mauritiana, I. palmata* and *I. batatas*). The results indicated that the phytochemical constituent of *I. mauritiana viz*., undecane, quinic acid which is to have insecticidal activity. The major constituent of *I. batatas* comprises of melezitose (38.53%) and alpha-I-rhamnopyranose (21.26%). It can be concluded that the phytochemical constituents of *I. mauritiana* was responsible for the antibiosis. 

**KEYWORDS:** Antibiosis, bioactive, insecticidal, bioassay, undecane

## INTRODUCTION

The weevil *Cylas formicarius* F. belonging to Coleoptera, Brentidae, is a destructive pest of sweet potato and is widely spread throughout the tropical regions of the world, but the methods of control are the significant problem faced by growers in most countries producing sweet potato. Generally, weevils cause severe feeding destruction to sweet potato roots, vines, stems and leaves through their life cycle, beginning from the egg stage to adult stage. Weevil infested tubers are bitter due to the production of a terpene compound and the infested tubers are unfit for consumption or convert to livestock, resulting major economic losses (Uritaini *et al.,*1975; Palaniswami and Mohandas, 1993; Korada *et al*., 2010a; Kyereko *et al.,* 2019). Although *C. formicarius* prefers sweet potato,

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more than 30 species of *Ipomoea* and other genera have been recorded as its host plants (Sutherland, 1986; McConnell and Hossner, 1991). About 500-600 species were included in the genus *Ipomoea* sp. within the Family Convolvulaceae (Austin and Huáman, 1996). Studies have proved that the management sweet potato weevil (SPW) can be done by integrated pest management *viz.,* removal and destruction of hosts, cultural methods, biological control, botanicals, chemical pesticides, tolerant varieties and use of semiochemicals (Palaniswami *et al.,* 1992; Pillai *et al.,* 1993; Palaniswami and Chattopadhyay, 2006; Korada *et al.,* 2010a).

Earlier studies on the identification of the resistant sweet potato genotypes to the weevil indicated only relatively tolerant ones. Studies conducted at AVRDC and Penghu Island has reported screening of the population *I. trifida* x *I. batatas* hybrids with high yield and low weevil infestation (Talekar, 1987). An indigenous cultivar Selopia was identified moderately resistant to the weevil by screening based on crown damage grade index (DGI), percentage tuber damage, tuber DGI, adult emerged per kg infested tuber (Palaniswami and Mohandas 1992). Korada *et al*. (2010b) reported that among the sweet potato genotypes, *viz*., Goutam, Sourin, Gouri and CIP-6 evaluated for SPW resistance, CIP-6 was the most susceptible. Further in their electroantennogram studies identified the electrophysiological response of female antenna to the volatile extracts of aerial plant parts and roots was higher than the male antenna of the weevil. In olfactometer studies, the headspace volatiles of genotype CIP-6 attracted more number of female *C. formicarius* weevils than volatiles of Gouri, Goutam and Sourin. Variation in the preference of sweet potato genotypes to *C. formicarius* is attributed to differential emission of volatiles from the aerial parts and roots. Reddy *et al*. (2015) reported that the weevil, developed faster on *Ipomoea batatas* than on *I. triloba.*

Anyanga *et al*. (2013) found that hydroxycinnamic acid esters on the exterior and the root latex, decreases weevil's nourishment and oviposition providing resistance to SPW. Okada *et al*. (2019) identified genetic regions associated with weevil resistance in 90IDN-47 and PSL sweet potato genotypes by genome wide association studies (GWAS) in Japan. In their experiment on the degree of weevil damage to the genotypes, no single nucleotide polymorphisms (SNPs) were identified above the significance thresholds. However, one relatively high peak was found in the 90IDN-47 genotype, which showed resistance to weevils. On the other hand, one relatively high peak was also detected in the PSL genotype, which showed susceptibility to weevils. These results suggest that two regions could affect weevil resistance and may contain the gene(s) controlling weevil resistance. SPW can survive on average longer than four months on sweet potato as well as *I. triloba* (Reddy and Chi, 2015). Hence identification of host plant resistance source against weevil is one of the alternative strategies for the pest management. In the present study, genotypes from different species of *Ipomoea* were selected based on the reports (Reddy and Chi, 2015) on host preference by weevils and experiments were conducted to screen wild *Ipomoea* spp. for resistance against weevil based on the nature of feeding by sweet potato weevils and their phytochemical constituents.

## MATERIALS AND METHODS

**Multiple choice bioassay:** Multiple choice bioassays (Vos and Jander, 2008) were carried out using leaves, vines and roots of plant species *viz Ipomoea batatas, I. mauritiana*, *I. palmata, I. obscura* and *I.triloba* Five plant samples were placed in large Petridish (180x30mm) and 20 weevils (@1male: 5females) were released in the centre of the Petridish. The sweet potato weevils were reared in Entomology laboratory and were used for bioassay. The position and readings of feeding holes by weevils (cumulative values) were recorded for three consecutive days. Experiment using leaves, vines and roots were conducted separately in three replications and each experiment was repeated thrice. Control samples (leaves, vines, roots) for each experiment were kept separately.

**Two - choice bioassay:** No-choice bioassays (Vos

and Jander 2008) were carried out using fresh leaves, vines and roots of sweet potato and *I. mauritiana.* The plant samples were placed in large Petridish (180x30mm) and 18 sweet potato weevils (1male: 5 females) were introduced to the centre of the Petridish. The sweet potato weevils were reared in Entomology laboratory and were used for bioassay. The position and readings of feeding holes by weevils (cumulative values) were recorded for three consecutive days. Experiment using leaves, vines and roots were conducted separately in three replications and each experiment was repeated thrice. Control samples (leaves, vines, roots) for each experiment were kept separately.

Data were subjected to analysis of variance using IBM SPSS version 21.The differences between the treatments was measured by tuckeys test at *P*_0.05, and the treatment means were compared using the least significant difference at 5 per cent. Data for no choice assay were subjected to t-test at *P*_0.05.

**Gas chromatography-Mass spectrum analysis:** Further for GC-MS analysis one tuberous wild *I. mauritiana*, one non-tuberous wild *I. palmata* and *I. batatas* were selected for the anlaysis. The required quantity of the whole plant tubers/roots was washed, air dried and weighed. It was transferred to a flask, treated with methanol of 500ml until the tubers was fully immersed, incubated overnight and filtered through a Whatmann No. 41 filter paper**.** Before filtering, the filter paper along was wetted with methanol. The filtrate is then concentrated to 5 ml using flash evaporator. The GC-MS analysis was done at Sophisticated Analytical Instruments Facility (SAIF), IIT, Chennai. GC-MS analysis of the methanol extract was performed using an Agilent-Technologies 8890 Network GC system equipped with an Agilent-Technologies 5977 mass selective detector (Agilent-Technologies, Little Falls, CA, USA). For MS detection, the electron ionization mode with ionization energy of 70 eV was used, with a mass range at m/z 50–600. An HP-5MS capillary column (30 m × 250 ìm, film thickness 0.25 ìm) was used for GC/MS. The column temperature was programmed from 180 to 300 °C at a rate of 5 °C/min with the lower and upper temperature being held for 3 and 5 min, respectively. GC was performed in the split mode. Helium was used as carrier gas at a flow rate of 1.2 ml/min. An injection 1 µl was used for each diluted extract. Essential compounds were identified by their retention times and mass fragmentation patterns using data of standards at NIST library

## RESULTS AND DISCUSSION

**Multiple choice bioassay:** The weevil feeding holes on *I. mauritiana* was significantly less compared with *I. batatas*, *I. triloba. I. palmata* and *I. obscura*. The insect feeding holes on *I. mauritiana* leaves (1.67+1.52) was significantly low, when compared tpother *Ipomoea* species (Table 1). Similarly the same pattern was observed for the three consecutive days and mortality of insects was also observed. The insect feeding holes on *I. mauritiana* vines was less (7.67±2.96), compared to other *Ipomoea* species. The same pattern was observed for the three consecutive days given (Table 2). The insect feeding holes on *I. mauritiana* tubers was significantly low (12.67 ± 2.30), when compared to other *Ipomoea* species (Table 3).

**Two-choice bio-assay:** The weevil feeding holes on leaf, vines and tubers of *I. mauritiana* and *I. batatas* indicated great variation between them. *I. mauritiana* showed resistance to the weevil (Table 4).

Table 1. Leaf feeding (no. of holes) by the weevils on *Ipomoea* species in multiple choice bioassay

| Species | 1st Day | 2nd Day | 3rd Day |
|---|---|---|---|
| *I. mauritiana* | 1.67± 1.52[a] | 4.67±1.15[a] | 7.33±0.57[a] |
| *I. triloba* | 4.00± 1.00[a] | 7.00± 1.00[a] | 9.67± 0.57[a] |
| *I. palmata* | 10.00 ± 2.64[b] | 15.33± 2.30[b] | 18.33± 2.88[b] |
| *I. obscura* | 10.33± 2.08[b] | 14.67± 0.57[b] | 17.67±1.155[b] |
| *I. batatas* | 7.20± 4.10[b] | 17.00± 1.73[b] | 19.00± 1.00[b] |

Mean values (mean+standard p_0.05) represent error of feeding holes (cumulative) by sweet potato weevil on different *Ipomoea* sp. leaves

Table 2. Vine feeding (no. of holes) by the weevils on *Ipomoea* species in the multiple choice bioassay

| Species | 1st Day | 2nd Day | 3rd Day |
|---|---|---|---|
| *I. mauritiana* | 7.67± 2.96$^{ab}$ | 10.56± 3.37$^{ab}$ | 14.89± 2.14$^{b}$ |
| *I. triloba* | 4.22 ± 0.50$^{b}$ | 9.56 ± 1.38$^{b}$ | 12.00 ± 1.19$^{b}$ |
| *I. palmata* | 12.67 ±3.18 $^{a}$ | 18.00 ±3.46 $^{a}$ | 26.44±1.01$^{a}$ |
| *I. obscura* | 8.78 ± 1.50$^{ab}$ | 14.22± 3.65$^{ab}$ | 16.44 ± 2.14$^{b}$ |
| *I. batatas* | 11.22± 1.16$^{a}$ | 17.78± 2.41$^{a}$ | 24.22± 2.79$^{a}$ |

Mean values (mean+standard, *p*_0.05) represent the feeding holes (cumulative) by sweet potato weevil on different *Ipomoea* sp vines

Table 3. Tuber feeding (no. of holes) by the weevils on *Ipomoea* species in the multiple choice bioassay

| Species | 1st Day | 2nd Day | 3rd Day |
|---|---|---|---|
| *I. mauritiana* | 12.67 ± 2.30$^{a}$ | 22.67± 6.02$^{a}$ | 30.33 ± 2.51$^{a}$ |
| *I. triloba* | 10.67 ± 3.05$^{a}$ | 22.67 ± 8.73$^{a}$ | 34.33± 4.93$^{a}$ |
| *I. palmata* | 12.00 ± 0.00$^{a}$ | 26.67± 10.40$^{a}$ | 31.33± 5.50$^{a}$ |
| *I. obscura* | 12.67 ± 1.15$^{a}$ | 27.33± 3.51$^{a}$ | 30.67 ± 3.21$^{a}$ |
| *I. batatas* | 19.00 ± 2.64$^{b}$ | 31.00± 1.00$^{a}$ | 37.33± 2.08$^{a}$ |

Mean values (mean+standard, *p*_0.05) represent the feeding holes (cumulative) by sweet potato weevil on different *Ipomoea* sp roots

Table 4. Weevil feeding (no. of holes) on *Ipomoea* species in the two choice bioassay

| Species | 1st Day | 2nd Day | 3rd Day |
|---|---|---|---|
| Leaves | | | |
| *I. mauritiana* | 5.67±2.51 | 8.67±1.15 | 13.00±1.00 |
| *I. batatas* | 16.00±2.00 | 18.33±1.52 | 26.67±2.88 |
| Vines | | | |
| *I. mauritiana* | 11.00±1.00 | 13.00±1.73 | 14.67±2.51 |
| *I. batatas* | 16.33±0.57 | 19.00±2.64 | 25.67±1.15 |
| Tubers | | | |
| *I. mauritiana* | 6.33±0.57 | 12.33±2.51 | 17.67±1.52 |
| *I. batatas* | 28.33±7.63 | 35.00±5.00 | 43.00±4.58 |

Mean values (mean+standard, *p*_0.05) represent the feeding holes (cumulative) by the weevil

**Gas chromatography-Mass spectrum analysis:**

In all the multiple choice as well as two choice bioassay the feeding of weevils was significantly less in *I. mauritiana* which may be due to the presence of various phytochemical constituents. This shows the non-preference of the weevils always depends on the nature of host plant. GC-MS analysis of methanol extract of samples revealed phytochemical compounds, its retention time (RT) and peak area (%). The bioactivity of the identified compounds reported are presented along with its reference (Table 5). The phytochemical constituent of *I. mauritiana* include compounds undecane and quinic acid which are reported to have insecticidal activity whereas sucrose reported to enhance insecticidal activity**.** The most prevailing compounds identified in *I. mauritiana* were sucrose (77.01%), quinic acid (20.93%) whereas in *I. batatas* they were melezitose (38.53%) and alpha-I-rhamnopyranose (21.26%).

Higher levels of octadecyl and hexadecyl esters of hydroxycinnamic acids were identified in the root surface and root latex of sub-saharan sweetpotato variety, New Kawogo, contributing resistance to sweet potato weevil (Stevenson *et al.,* 2009). Anyanga *et al.* (2013) reported that the these compounds in high concentrations on root surfaces was strongly associated with resistance against adult oviposition and feeding. They reduce the development of sweet potato weevil larvae and suggested that differences in the concentration of these compounds between varieties explain differences in resistance. Among the five *Ipomoea* species the weevil infestation was significantly less in *I. mauritiana*. Phytochemical screening of methanolic extract revealed the presence of various compounds which are reported to have insecticidal activity. These components might be responsible for the low weevil infestation in *I. mauritiana.*

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Table 5: List of the phytochemical compounds detected from the methanol extract of *Ipomoea* species through GC–MS analysis

| No | R/T | Peak % | Compound | Bioactivity | Reference |
|---|---|---|---|---|---|
| *I. mauritiana* | | | | | |
| 1. | 6.15 | 2.06 | Undecane *Ludwigia stolonifera* | Constituent of | Baky *et al.,* 2021 |
| 2. | 14.08 | 77.01 | Sucrose | Insecticide activity | Ezhilan and Neelamegam, 2012 |
| 3. | 17.97 | 20.93 | Quinic acid | Insecticidal activity | Li *et al.,* 2021 |
| *I. batatas* | | | | | |
| 1. | 5.68 | 3.48 | D-Alanine, N-proparglyoxy carbonyl-decyl ester | Constituent of *Averrhoa bilimbi* | Suluvoy and Grace *et al.,* 2017 |
| 2. | 6.85 | 2.26 | DL-Arabinose | Antimicrobial activity | Mohammed *et al.,* 2018 |
| 3. | 6.92 | 2.04 | 2-Deoxy-2-fluoro-1,6-anhydro -β-d-glucopyranose | Constituent of *Alternaria alternata* | Kamal *et al.,* 2015 |
| 4. | 7.13 | 2.03 | 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl | Antifungal activity | Teoh and Don, 2015 |
| 5. | 9.07 | 3.28 | 5-Hydroxymethylfurfural | Insecticidal activity | Chuang *et al.,* 2018 |
| 6. | 9.66 | 7.03 | 5-O-Methyl-d-gluconic acid dimethylamide | Antimicrobial, antioxidant | Kazi and Gude, 2022 |
| 7. | 10.24 | 2.19 | Octanamide, N-(2-mercaptoethyl) | Secondary metabolite of *Vitis vinifera* | Kadhim *et al.,* 2017 |
| 8. | 12.40 | 2.77 | Methyl 4-nitrohexanoate | Constituent of *Hugonia mystax* | Vasuki *et al.,* 2022 |
| 9. | 14.60 | 38.53 | Melezitose | Insecticidal activity | Gore and Schal *et al.,* 2004 |
| 10. | 18.45 | 4.04 | Desulphosinigrin | Antibacterial activity | Olajuyigbe *et al.,* 2018 |
| 11. | 26.38 | 1.97 | 1H-Benzocyclohepten-7-ol, 2,3,,4,4a, 5,6,7,8-octahydro-1,1,4a | Floral volatile constituents of *Crataeva religiosa* | Sharma *et al*., 2018 |
| 12. | 27.04 | 1.83 | Spiro[4,5]decan-7-one,1,8 -dimethyl-8,9-epoxy-4-isopropyl | Anti-inflammatory | Subin and Jagathy 2017 |
| 13. | 28.22 | 0.74 | Santamarine | Natural antioxidant with anti-photoaging | Oh *et al.,* 2021 |
| *I. palmata* | | | | | |
| 14. | 6.48 | 1.28 | Maltol | Mosquito larvicidal activity | Rajamanikyam *et al.,* 2017 |
| 15. | 9.41 | 1.28 | 4-Methylmannitol | Constituent of khat leaves | Alsanosy *et al.,* 2020 |
| 16. | 10.53 | 1.77 | 2H-Pyran-2-onetetrahy dro-6-propyl- | Fatty acid composition of *Trichosanthes cucumerina* bio-oil | Manimaran *et al.,* 2020 |

| | | | | | |
|---|---|---|---|---|---|
| 17. | 13.88 | 3.29 | Panaxydol | first isolated from roots of P. ginseng induces apoptosisin cancer cells | Takahashi *et al.,* 1964; Kim *et al.,* 2016 |
| 18. | 14.00 | 2.56 | Melezitose | Insecticidal activity | Gore and Schal *et al.,* 2004 |
| 19. | 18.07 | 7.65 | Quinic acid | Insecticidal activity | Li *et al.,* 2021 |
| 20. | 23.39 | 2.33 | 3-(6,6-Dimethyl-5-oxohept-2-enyl)-cycloheptanone | Constituent of *Myoporum bontioides* | Minh et al., 2020 |
| 21. | 26.56 | 5.22 | Scopoletin | Antitermite activity | Adfa *et al*., 2010 |
| 22. | 27.04 | 2.59 | 1,8-Naphthalenedione, 8a-ethylperhydro | Cconstituent of *Plectranthus hadiensis* | Sripathi *et al.,* 2017 |

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# Synthesis and characterization of silver nanoparticles using *Datura metel* L. (Solanaceae) leaf extract and its larvicidal activity on *Epilachna vigintioctopunctata* F.

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**ABSTRACT:** Insecticidal activities of synthesized silver nanoparticles of leaf extract of *Datura metel* L. (Solanaceae) (DM) against grubs *Epilachna vigintioctopunctata* F. (Coleoptera, Coccinellidae) at varying levels of concentrations was evaluated. DM leaf extract was used to create AgNPs, and nanoparticle production could be seen after six hours. UV-vis spectrophotometer, Particle size analyzer, FTIR and SEM analysis were used to confirm the synthesis of AgNPs. GCMS spectra of leaf extract of DM showed 20 substances, of which nine were known s phytochemicals and the others were unidentified. UV-visible spectra to analyse the Surface Plasmon Resonance for AgNPs revealed in the range of 366 - 374nm. $LC_{50}$ values for the AgNPs synthesized leaf extracts calculated 24 h after treatment against the fourth instar larvae of *E. vigintioctopunctata* using probit analysis revealed the $LC_{50}$ of the aqueous leaf extract as 252.31ppm and that of AgNPs synthesized leaf extract as 396.09ppm. When comparing the aqueous and AgNPs synthesized leaf extracts, AgNPs nanoparticles synthesized leaf extracts were more efficient as larvicide than aqueous leaf extract. 

**KEY WORDS:** AgNPs, aqueous leaf extract, larvicidal activity, $LC_{50}$ values

## INTRODUCTION

A common and well-liked vegetable crop growing in the subtropics and tropics is eggplant, *Solanum melongena* L. (Sarkar *et al.,* 2006) and an important vegetable crop in India. According to their size, shape, and color, there are numerous varieties of brinjal available in India (Nisha *et al.,* 2009). The significant losses caused by damage to different agricultural crops are largely attributable to insect pests. According to estimates, insect pests are responsible for 23 per cent of all agricultural losses (Agarwal, 2011). Pesticides are used often and to control these pests in the vegetable fields, which has led to widespread resistance development, unfavorable impacts on non-target organisms, the presence of hazardous residues in food, and environmental and health risks (Kranthi *et al*., 2002). All these have highlighted the requirement to create unique, risk-free, and environmentally friendly pest management methods. *Epilachna vigintioctopunctata* F. (Coleoptera,

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Coccinellidae) is a serious pest of solanaceous crops, especially potatoes and aborigines, throughout its range. Adult and grubs feed on the surface of leaves by scraping away the surface cells between the main veins to leave irregular-shaped holes or strips. Heavy feeding damages the leaves, giving them a skeletonized or lace-like appearance. The damaged leaves turn brown and curl as they dry before falling off. *Datura metel* L. (Solanaceae) is a shrub-like, commonly found in India and has pesticide and medicinal properties. An attempt was made to investigate *D. metel* in the creation of silver nanoparticles from its leaf extract and its efficiency as larvicide at varying concentrations on *E. vigintioctopunctata*.

## MATERIALS AND METHODS

Leaves of *D. metel* were gathered in and around Meenampatti, Sivakasi Taluk, between July 2021 and March 2022. The study region is located between latitudes of 9°27'2.6424" from the north and longitudes of 77°48'26.0496" from the east. *D. metel* leaves plucked were cleaned in tap water and allowed to air dry for 5-7 days in the shade. Using an electric blender, the air-dried plant components were powdered. For extraction, 10g of fine leaf powder was collected in a beaker with 100ml of double-distilled water that had been sterilized. After that, it was heated at $60^0$C for 3 number 1, and the extract was kept at -20°C and used within a week.

**Phytochemical analysis:** Agilent GC 7890A/ MS 5975C and a capillary column Agilent DB5MS were used in the GCMS to evaluate the *D. metel* leaf extract. The mass spectrometer was tuned to 70 eV, and the computer library created by WILEY7, NIST05, and NIST05s was used to identify unknown substances using probability-based matching.

**Synthesis of silver nanoparticles from leaf extract:** The precursor used to create silver nanoparticles was silver nitrate. Analytical-grade silver nitrate ($AgNO_3$) in the amount of 16.961mg was measured out and mixed with 90ml of Milli-Q water. In a l Erlenmeyer flask, 90ml of produced 1mM aqueous $AgNO_3$ solution was added to 10ml of aqueous leaf extract and incubated at room temperature in the dark. AgNPs were being produced as a result of the transition from light yellow to dark brown (Lingarao and Savithramma, 2013).

**Characterization of nanoparticles:** Utilizing UV-Visible spectroscopy, the initial characterization of AgNPs was completed. Synthesized silver nanoparticles were subjected to FTIR analysis, particle size analysis and SEM.

**Epilachna beetle rearing:** A mass culture of *E. vigintioctopunctata* was kept in a lab at the Department of Zoology, Ayya Nadar Janaki Ammal College, Sivakasi, Tamilnadu, India, using brinjal leaves as feed, in order to consistently obtain a large number of epilachna larvae for experimental usage. The *E. vigintioctopunctata* infested brinjal plant was grown in the nearby agrifarm of Sivakasi, where the adults of *E. vigintioctopunctata* were collected. The laboratory reared epilachna larvae were used for bioassays and the cultures were maintained throughout the study period. The adults were allowed to breed in the laboratory condition, when the larvae hatch from the egg they were fed with fresh brinjal leaves. They were permitted to develop till becoming third instar stages, and then were used to assess the larvicidal efficiency.

**Larvicidal activity:** The leaf extract's larvicidal activity was tested in the laboratory settings. By dilution with de-ionized water, various amounts of aqueous and synthetic silver nanoparticle leaf extract were created. Each extract had a concentration of 100, 50, 25, 12, 5 and 6.25 mg ml-1. To examine leaf extract's larvicidal potential, a spray test was conducted. Different concentrations of extracts were made, sprayed on brinjal leaves, and given to epilachna beetle larvae as feed. To determine the larvicidal activity of each concentration of extracts, ten larvae per concentration were subjected and replicated thrice. Similar to this, three replicates of control group (tap water) for each test was done. After a 24h exposure period, the mortality rate was observed. For each concentration, the percentage of larval mortality from the replicates was calculated.

**Statistical analysis:** Larval mortality analysis assessed using standard deviation and mean separation. Using the program SPSS, 2007, the probit analysis for $LC_{50}$, the upper and lower 95 per cent confidence bounds were determined.

## RESULTS AND DISCUSSION

Aqueous *D. metel* leaf extract was used to perform green production of silver nanoparticles. The initial confirmation of the biosynthesis of silver nanoparticles using aqueous leaf extract was the visible observation of colour change. The initial colour of the leaf extract suspension was pale yellow; but, after the addition of silver nitrate and an overnight incubation at room temperature, the colour changed to brown (Fig. 1).

**GCMS analysis of leaf extract:** As many as 20 compounds were detected in GCMS spectra of *D. metel* leaf extract, of which nine were recognized. The remaining 12 substances were unidentified. Retention times, mass spectra, and a library of typical compounds were compared to determine whether phytochemicals were present. Among the nine compounds, L-Arabinitol ($C_5H_{12}O_5$) had 40.57 per cent peak area, with a retention time of 4.887 minutes, having a molecular weight of 152.15 g $mol^{-1}$. The remaining compounds were found in the range of 1 – 7 per cent peak area (Table 1).

**Characterization of leaf extract:** The Surface Plasmon Resonance (SPR) is analyzed using an ultraviolet-visible spectrum. AgNPs UV-visible spectra fell between 366 and 374 nm (Fig. 2a).

**Fourier Transform-Infrared (FTIR) analysis:** FTIR analysis showed the vibrational spectra of AgNPs synthesized leaf extract (Table 2). The alcohol molecule found in the extract was responsible for distinctive peak that occurred at 3453.31. The peak at 760.87 was assigned to the stretching vibration C-CL group. The alkenes group is present in the AgNPs produced extract as indicated by the 1385.76 and 1639.38. Presence of aromatic compound is established by the peat at 1512.09. Alkanes group presence is confirmed by the absorption peaks at 2063.69and 2885.31 by C-H stretch and C-H stretch respectively. The peak at 2309.6 indicates the presence of alkynes in the AgNPs synthesized extract with Ca C stretch in AgNPs synthesized leaf extracts (Fig. 2b).

**Particles size analysis:** Dynamic light scattering (DLS) was used to establish the intensity-weighted mean diameter (Z-average) of the particle size of DM-AgNPs in the range of 0.5-1nm (Fig. 2c). The particle size and shape of the biosynthesized DM-AgNPs are also determined using SEM examination. The SEM investigation revealed that the produced nanoparticles' mean particle diameters ranged from 20 to 27nm (Fig. 2d).

**Larvicidal activity:** $LC_{50}$ values for the AgNPs synthesized leaf extracts calculated 24 h after treatment against the fourth instar larvae of *E. vigintioctopunctata* using probit analysis revealed the $LC_{50}$ of the aqueous leaf extract as 252.31ppm and that of AgNPs synthesized leaf extract as 396.09ppm. Larval mortality improved with

Table 1. GCMS spectra of identified phytochemicals from the extract of *Datura metel*

| No | RT (Min) | Compound | Peak area (%) | Molecular formula | Molecular weight ($g \cdot mol^{-1}$) |
|---|---|---|---|---|---|
| 1 | 4.887 | L-Arabinitol | 40.57 | C5H12O5 | 152.15 |
| 2 | 5.542 | Propanamide | 3.41 | $C_3H_7NO$ | 73.095 |
| 3 | 5.709 | Benzeneacetaldehyde | 7.09 | $C_8H_8O$ | 120.1485 |
| 4 | 7.398 | Benzoic acid | 1.22 | $C_7H_6O_2$ | 122.12 |
| 5 | 8.464 | Silane | 4.77 | $H_4Si$ | 32.117 |
| 6 | 11.264 | o-Cyanobenzoic acid | 1.98 | $C_8H_5NO_2$ | 147.130 |
| 7 | 12.230 | Pinacolyl ethyl phosphonofluoridate | 2.31 | $C_8H_{18}FO_2P$ | 196.199 |

Table 2. Functional groups detected in AgNPs synthesized leaf extract of *D. metel* as revealed by FTIR

| No | Absorption ($cm^{-1}$) | Class of compounds | Bond |
|---|---|---|---|
| 1 | 743.51 | Alkyl halide | C-CL stretch |
| 2 | 1385.76 | Alkenes | C-H bend |
| 3 | 1512.09 | Aromatic | C=C stretch |
| 4 | 1639.38 | Alkenes | C=C stretch |
| 5 | 2063.69 | Alkanes | C-H stretch |
| 6 | 2309.60 | Alkynes | Ca=C stretch |
| 7 | 2885.31 | Alkanes | C-H stretch |
| 8 | 3453.31 | Alcohol | O-H stretch |

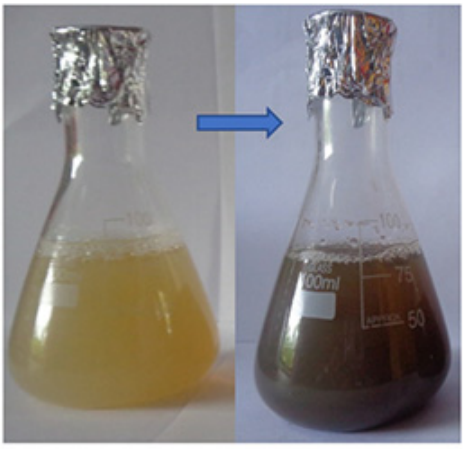

Fig. 1 Visible colour change of *Datura metel* leaf extract after sivler nanoparticles synthesis

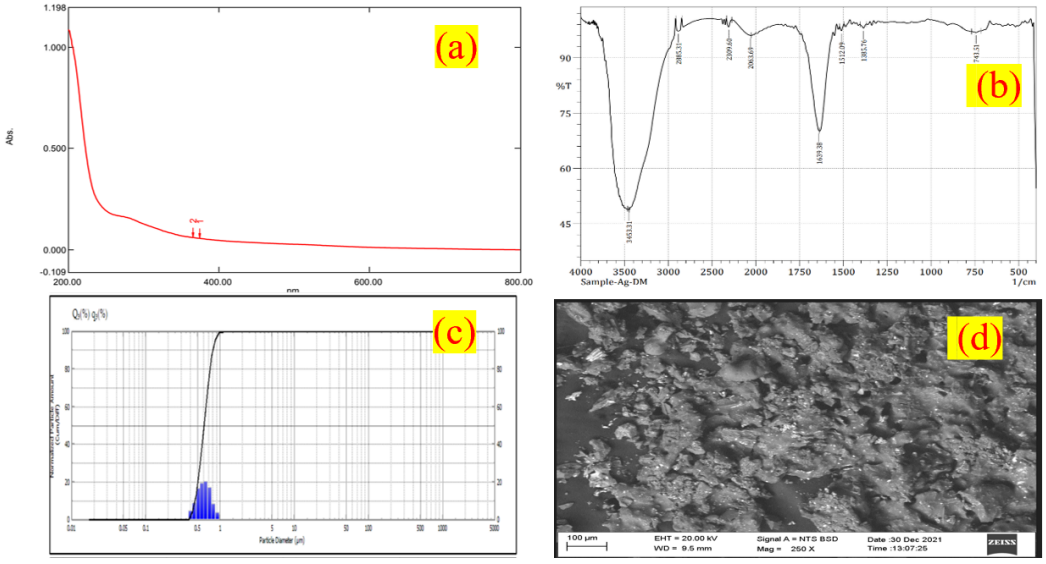


Fig. 2 Characterization of Silver Nanoparticles synthesized (a) UV-Visible Spectral analysis; (b) FTIR analysis; (c) Particle size analysis; (d) SEM analysis

increase in the concentration of silver nanoparticles synthesized leaf extract (Table 3). AgNPs synthesized leaf extract @100 concentration recorded 80 per cent mortality; at the same the aqueous leaf extract showed 10 per cent mortality. When comparing the aqueous and AgNPs synthesized leaf extracts, AgNPs nanoparticles synthesized leaf extracts were more efficient than aqueous leaf extract as larvicide.

Due to the pressing needs to create ecologically friendly technology, biosynthesis of nanoparticles has received a lot of attention. A significant method for creating many types of nanoparticles, such as copper, iron, platinum, silver, and zinc, has been the biosynthesis of nanoparticles utilizing biological agents (Rasheed *et al.,* 2017; Sharon *et al*., 2018). Bioresearch in the domain of explaining the mechanism of plant-mediated nanoparticle production holds great promise (Kumar and Yadav, 2009). The synthesis of AgNPs using *D. metel* was verified in the current work utilizing a variety of cutting-edge methods. When *D. metel* leaf extract is added to the silver nitrate solution, the mixture changes color from pale yellow to brown due to the reduction of the silver ion, indicating the creation of silver nanoparticles. The current work provides proof that the extract of *D. metel* has a potential to

Table 3. Silver nanoparticles synthesized leaf extract on the mortality of IV$^{th}$ instar larvae of *E. vigintioctopunctata* (@10 larvae/ conc)

| Concentration | Mean mortality of IV$^{th}$ instar larvae in | |
|---|---|---|
| | aqueous | AgNPs |
| 6.25 | 0 | 1 |
| 12.5 | 0 | 2 |
| 25 | 0 | 2 |
| 50 | 0 | 5 |
| 100 | 1 | 8 |

reduce silver ions (Ag+ into Ag0) and convert silver nitrate to silver nanoparticles while also having larvicidal effects on epilachna beetles. Similar research on *Polyalthia longifolia* samples, whose colour ranges from nearly colorless to brown, was published by Kaviya *et al.,* in 2011.

By comparing mass spectra, retention times, and a library of common compounds, it was possible to confirm that leaf extract contained phytochemical components. In GCMS spectra of *D. metel* leaf extract 20 compounds were detected, of which nine were recognized as phytochemicals. Mishra and Patnaik (2020) obtained comparable findings from a methanol extract of the complete *Withania somnifera* plant. According to the size and polydispersity of NPs, the distinctive peak of AgNPs is located about 430nm (Anandalakshmi *et al.,* 2016). According to Rajagopal *et al.* (2021), the UV-Vis spectra of the CuNPs produced using *Wrightia tinctoria* displayed absorption peak maxima at 357 nm. Plants create phytochemicals either through their primary or secondary metabolism. The majority of the time, they are biologically active in the plant host and aid in plant growth or defense against pests, diseases, or predators.

Measurements using the Fourier transform infrared spectroscopy technique are used to pinpoint the potential biomolecules in charge of the reduction, capping, and effective stability of silver nanoparticles (Padalia *et al.,* 2015). It was demonstrated that NPs were generated by the presence of functional groups like alcohol, halides, alkanes, alcohol, and aromatic compounds (Prabha *et al.,* 2022).

The larvicidal activity of silver nanoparticles made from *D. metel*'s aqueous extract against the epilachna beetle was observed in this work. The $LC_{50}$ values calculated in the present study revealed that both aqueous and AgNPs have remarkable larvicidal effect on epilachna beetle. According to Islam *et al.* (2011), the $LC_{50}$ values for three medicinal plants were 18.40, 23.70, and 29.61 per cent, for the epilachna phytophagous pest. When bioefficacy of two indigenous plant products, namely seed extracts of *Strychnos nuxvomica* and *Pachhyrrhizus erosus*, and two entomopathogenic fungi, *Beauveria bassiana* and *Metarrhizium anisopliae*, were tested against the epilachna beetle on bottle gourd, the population of the epilachna beetle was significantly reduced (Vishwakarma *et al.,* 2011). According to Ahmed (2007), spraying the castor plant *Ricinus communis* aqueous extract on sunflower, *Helianthus annulus* foliage and capitula, reduced epilachna attacks resulting improved the oil seed harvest.

The present findings clearly show that at certain levels, the suggested green silver nanoparticles have an effect on *E. vigintioctopunctata* that causes mortality. Ag+ ions are produced from the nanoparticle's surface by oxidation processes, and when they enter an insect's physiological processes, they interact with biological components (such as insect proteins) and induce toxicity (Park *et al.,* 2010). AgNPs are also known to bind with thiol groups in proteins and promote their denaturation, which causes the death of larvae (Johnston *et al.,* 2010). Hence, *D. metel*'s aqueous leaf extract and silver nanoparticles produced leaf extract can be effectively used as larvicide to *E. vigintioctapunctata*.

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# Redescription of *Carbula indica* (Westwood, 1837) (Hemiptera, Heteroptera, Pentatomidae) from West Bengal, India with a key to the Indian species of the genus *Carbula* Stål, 1865

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**ABSTRACT**: *Carbula indica* (Westwood, 1837) (Hemiptera, Heteroptera, Pentatomidae) re-described with illustrations based on the material collected from the Himalayan hills of Darjeeling, West Bengal, India. New characters are included, along with both male and female genitalia and morphometric measurements to facilitate easy species determination. A key to the Indian species of *Carbula* Stål, 1865 is also presented. © 2023 Association for Advancement of Entomology

**KEYWORDS:** Taxonomy, morphometrics, genitalia, species determination, key

## INTRODUCTION

The genus *Carbula* Stål, 1865, is distributed in the Oriental and Afrotropical regions, represented by 76 species worldwide, of which ten species are known from India (Ravneet Kaur *et al.*, 2013). *Carbula* can be recognized by the following characters: body dorsally ovate and ventrally convex; head rounded or somewhat truncated at apex; mandibular plates equal in length and pronotum with anterior lateral angles obtuse; genital capsule quadrangular and paramere with bilobed crown. Westwood (1837) placed *Carbula indica* under the genus *Pentatoma*, Distant (1902) later transferred it to the genus *Carbula* Stål. Both described the species based on external coloration and a few morphological features, which are insufficient to identify the species correctly. This species was reported from West Bengal (Darjeeling), Sikkim and Nepal by Distant (1902); later reported from China (Rider *et al.*, 2002). During a collection of Himalayan Pentatomidae, four specimens (2 males, 2 females) of *C. indica* from Darjeeling were found and this opportunity is taken to re-describe and illustrate the species, including male and female genitalia and measurements to facilitate easy species identification. A key to the Indian species of *Carbula* Stål, 1865 is also provided.

## MATERIALS AND METHODS

The material for the present study includes dry-pinned specimens housed at the Hemiptera Section of the Zoological Survey of India, Kolkata, West Bengal. The male genitalia was dissected following

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the method given by Ahmad (1986). After boiling the whole abdomen in hot water for about 10–15 minutes, the female genitalia was dissected with potassium hydroxide (KOH - 10%). The internal contents were cleared after thoroughly washing it in distilled water 2–3 times. With the help of fine forceps, the terminalia and spermatheca were detached from the abdominal ventrites. The measurements are given in millimeter and presented as median, with minimum and maximum values given in bracket. The following dimensions were measured: Body length (from apex of mandibular plates to apex of membrane, dorsal view), head length (from apex of mandibular plates to anterior margin of pronotum, dorsal view), head width (width of head including compound eyes, dorsal view), interocular width (between inner margins of compound eyes, dorsal view), length of each antennal segment, length of each segment of rostrum, pronotum length (medially, from anterior to posterior margin of pronotum, dorsal view), pronotum width (maximum width between humeri in dorsal view), scutellum length (medially from base to apex) and scutellum width (maximum width at base between basal angles of scutellum). Morphological terminology used for male and female genitalia broadly follows Salini (2019). The photographs were taken under a Leica M205A stereomicroscope using a Leica DMC-4500 camera. The photographs were processed in LAS V4.12 software for morphometry. Photographs were edited using Adobe Photoshop CS (Version 8.0).

**Abbreviations used:** Vlfs: Valvifers, Lt: Laterotergite, P: Paramere, PA: Processes of aedeagus, CP: Conjunctival Processes, PT: Phallotheca.

## RESULTS AND DISCUSSION

***Carbula indica*** (Westwood, 1837) [Figs. 1-16]

*Pentatoma indica* Westwood, 1837: 42.

*C. fusca* Distant, 1887: 346.

*C. indica*: Distant, 1902: 171.

**Type locality**: INDIA: West Bengal: Darjeeling: Kurseong

***Material examined*:** 2 male, 2 female, Lepchajagat, Darjeeling, West Bengal, 4.vi.1975, Coll. J. K. Jonathan.

**Redescription:**

***Coloration:*** Dark brownish yellow (Figs. 1, 12); anterior lateral margins of pronotum yellowish-brown; first three segments of antennae yellowish-brown, segment- IV slightly darker brown and apical two-third portion of last segment blackish. Abdomen pale brown with a broad central black fascia, spiracles and small marginal spots, black; rostrum blackish-brown with apex black; legs yellowish-brown with black punctation; genital capsule, pale brown and blackly punctate.

**Head:** Wider (across eyes) than long (1.911 : 1.503 mm) (Fig. 3); antennae five-segmented, segment IV longest, segment V longer than II, III, segment I smallest; rostrum long, four-segmented, passing metacoxae reaching up to II abdominal segment (Fig. 15) but sometimes up to anterior margin of III abdominal segment; segment II longest.

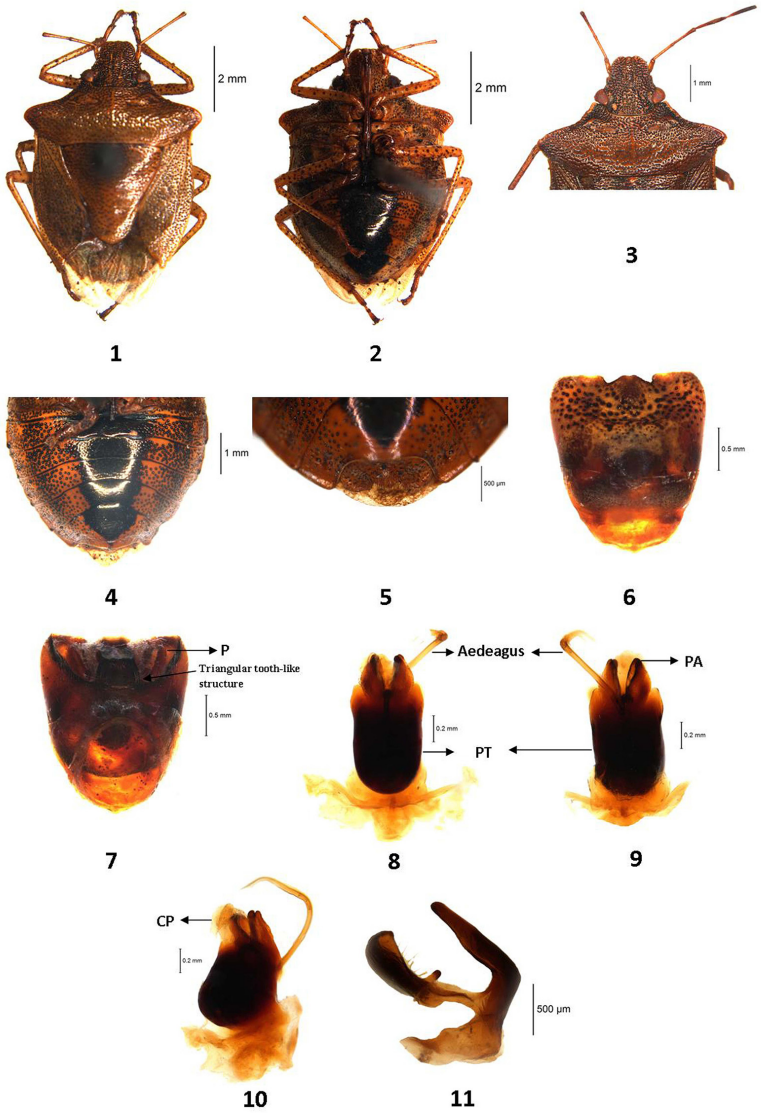
**Thorax:** Pronotum hexagonal , wider than longer, its width is 2.5 times than its length and about 2.5 times wider than head, anterolateral margins slightly serrated, lateral margins smooth, humeral angles rounded (Fig. 3).

**Scutellum**: Slightly longer than broad at base (3.747: 3.714mm) (Figs, 1, 10), apex rounded; hemelytral membrane passing tip of abdomen (Fig. 2).

**Legs:** With fine hairs and blackly punctate. Tibiae longer and slender than respective femora. Tarsi hairy, second tarsal segment shortest.

**Abdomen:** Abdomen broader than longer (6.396: 4.946) and with a broad central black fascia (Fig. 4).

**Male genitalia:** *Pygophore*. Longer than broad (2.136: 1.804mm), medially, in dorsal view (Fig. 7); postero-lateral lobes (= caudal lobes) tumescent (Figs. 5, 6), medial area slightly elevated (Fig. 5), subpentagonal in shape (Figs. 6, 7), Dorsal rim widely excavated with a narrow median notch

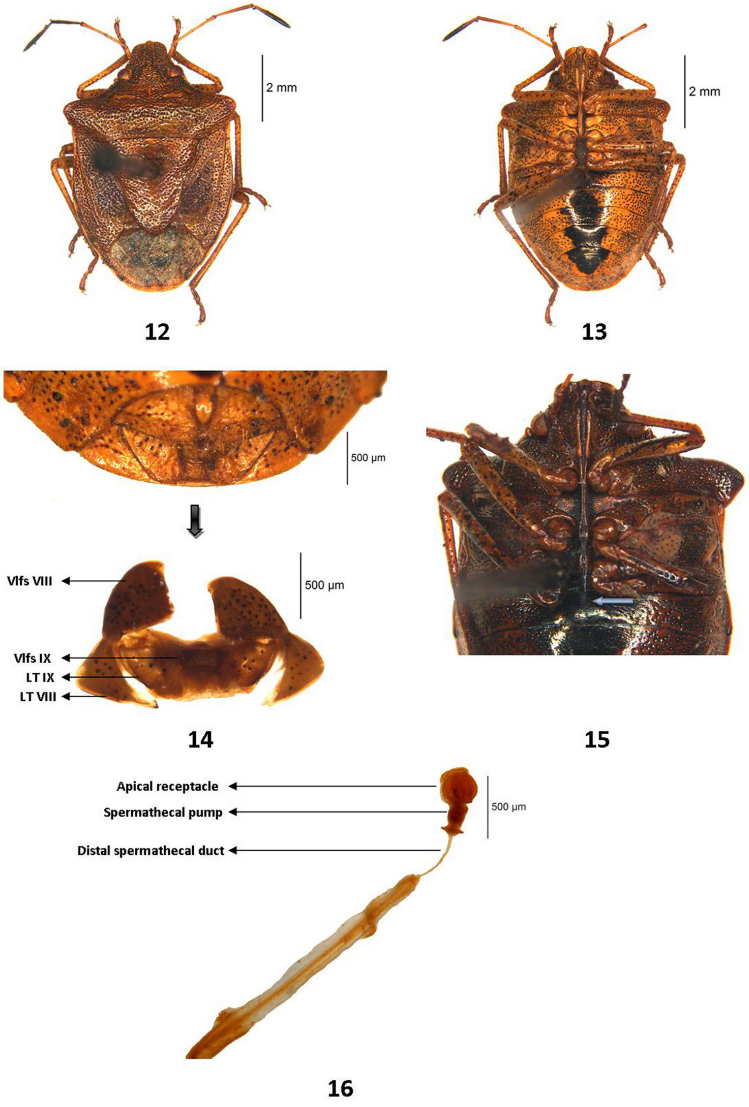



Figs. 1–11. Carbula indica (Westwood, 1837), male.1, dorsal; 2, ventral; 3, head; 4,abdomen; 5,apex of abdomn (ventral); 6, pygophore (ventral); 7, pygophore (dorsal); 8, phallus (dorsal); 9, phallus (ventral); 10, phallus (lateral); 11, paramere. P–Paramere, PA–Processes of aedeagus (=Penial lobes). CP–Conjunctival Processes, PT–Phallotheca.

which is associated with convex lobe-like outgrowth nearly resembling the 1+1 triangular tooth-like structure on the infoldings of dorsal rim (Fig. 7). There is a shallow roughly W-shaped notch at the medial portion of ventral rim (Fig. 6). *Phallus.* Phallotheca of phallus (Figs. 8, 9) is barrel-shaped, slightly sclerotized without any process; one pair of slightly sclerotized processes of aedeagus (=Penial lobes), encircled by transparent, membranous conjunctival processes (Fig. 10); adeagus long, tube-like and much longer than processes of aedeagus (=Penial lobes).

*Paramere*. Moderately sclerotized, crown bilobed (Fig. 11), upper lobe slender; lower lobe broader, spoon-shaped with a small projection at proximal end, slightly curved upwards facing towards upper lobe.

**Female genitalia:** Valvifers VIII sub-triangular with inner lateral margin curved (Fig.14); valvifers VIII ventrally not meeting each other; valvifers IX fused to single quadrate plate with posterior margin concave, laterotergite VIII posteriorly encompassing laterotergite IX (Fig.14);




Figs. 12–16. *Carbula indica* (Westwood, 1837), female. 12, dorsal; 13, ventral; 14, Terminalia; 15, rostrum; 16, Spermatheca. Vlfs–Valvifers, Lt–Laterotergite

spermatheca (Fig.16) with proximal spermathecal duct longer than distal spermathecal duct, median dilation present but not balloon-like; intermediate part of spermatheca (=spermathecal pump) short, only single flange (proximal flange) visible; apical receptacle large or-bicular, with a finger-like process.

**Measurements:**

**Males** (n = 2); median (minimum–maximum). Body length 7.050 (6.798–7.303); head: length 1.503 (1.496–1.510), width (across eyes) 1.911(1.897–1.925), interocular width 1.183 (1.177–1.189); lengths of antennal segments: I—0.280 (0.271–0.289), II—0.756 (0.747–0.765), III—0.728 (0.721–0.735), IV—0.931 (0.926–0.936), V—0.839 (0.833–0.845); lengths of segments of rostrum: I—1.549 (1.536–1.562), II—1.680 (1.600–1.761), III—0.800 (0.800–0.801), IV—0.668 (0.626–0.711); pronotum: length 1.942 (1.938–1.946), width: 4.879 (4.861–4.897); scutellum: length 3.276 (2.805–3.747), width (at basal angles) 3.247 (2.781–3.714).

**Females** (n = 2); median (minimum–maximum). Body length 7.367(7.359–7.375); head: length 1.693 (1.665–1.721), width (across eyes) 2.034 (1.956–2.112), interocular width 1.230 (1.155–1.251); lengths of antennal segments: I—0.358 (0.352–0.364), II—0.755 (0.745–0.765), III—0.767 (0.765–0.769), IV—0.936 (0.929–0.943), V—0.898 (0.885–0.911); lengths of segments of rostrum: I—1.585 (1.566–1.604), II—1.734 (1.707–1.762), III—0.796 (0.792–0.801), IV—0.642 (0.628–0.656); pronotum: length 2.107 (1.992–2.222), width: 5.420 (5.284–5.556); scutellum: length 3.304 (2.861–3.748), width (at basal angles) 3.297 (2.849–3.746).

**Distribution:** India: West Bengal (Darjeeling, Kurseong) and Sikkim. *Elsewhere*: Nepal (Distant, 1902) and China (Rider *et al.*, 2002).

**Key to species of *Carbula* Stål, 1865 from India**

1. Humeral angles spinously produced.....2

- Humeral angles obtusely angulate.....5

2. Upwardly and forwardly directed humeral angles.....3

- Slightly backwardly directed humeral angles with subacute spines...***biguttata*** **(Fabricius, 1794)**

3. Scutellum densely punctate, upper lobe of parameres slightly angulate apically ..... ***aliena*** **Distant, 1918**

- Scutellum sparingly and coarsely punctate, upper lobe of parameres apically nearly broadly rounded.....4

4. Scutellum with a Y-shaped luteous marking at middle portion and punctuate except each basal angle and the apex..... ***scutellata*** **Distant, 1887**

- Scutellum brownish-ochraceous without Y-shaped luteous marking and uniformely punctuate except two basal angle and apex ..... ***aspavia*** **Distant, 1908**

5. Mandibular plates equal to clypeus.....6

- Mandibular plates longer than clypeus .....***rugulosa*** **Distant, 1902**

6. Median continuous black stripe on each of the abdominal segments; rostrum passing metacoxae reaching up to II abdominal segment but sometimes up to anterior margin of III abdominal segment, humeral angle less produced with apices rounded .....***indica*** **(Westwood, 1837)**

- Median black stripe only on two terminal abdominal segments; rostrum just passing metacoxae reaching up to II abdominal segment, humeral angle broadly produced with apices obtuse and slightly paler and levigate .....7

7. Scutellum brownish-ochraceous, uniformely punctuate and without any luteous spots at each basal angle and apex ..***crassiventris*** **(Dallas, 1849)**

- Scutellum luteous, sparingly punctate with a large luteous spot at each basal angle and apex.....8

8. Antennae luteous.....***socia*** **(Walker, 1867)**

- Antenna with the first, second, and third joints ochraceous, fourth and fifth black with their bases ochraceous .....9

9. Antero-lateral margins of pronotum smooth, luteous levigate.....***producta*** **Distant, 1901**

- Antero-lateral margins of pronotum crenulated, luteous levigate .....***insocia*** **(Walker, 1868)**

The *C. indica* specimens were compared with the photographs of paratype of *C. indica* held by Natural History Museum, London, thus enabled us to redescribe the species in greater detail. *C. indica* is closely related to *C. crassiventris* on the basis of morphological data, during re-examination of the both species were found to have some minute morphological differences between these two species. Re-examination revealed that humeral angle of pronotum less protruded in *C. indica*; first three antennal segment of *C. indica* yellowish-brown, segment- IV slightly darker brown and apical two-third portion of last segment blackish, while in *C. crassiventris* whole segments of antennae reddish brown; in *C. indicia* adomen pale brown with a broad central black band, while in *C.*

*crassiventris* there was a more or less distinct central spot on each of the two terminal abdominal segments. It is important to note that this study will help to facilitate the taxonomic study of genus *Carbula* as the published information on this species is limited. Earlier work cited above only provided information on external coloration and a few morphological features, while this study has provided the details of morphology of this species, in the form of digital photographs of both male and female habitus and genitalia, for the first time.

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# Evaluation of the insecticidal action of polyphenolic compounds from *Streblus asper* (Lour.) on the red cotton bug, *Dysdercus cingulatus* (Fab.) (Hemiptera, Pyrrhocoridae)

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**ABSTRACT:** The study aims to evaluate oxidative stress and the activity of acetylcholinesterase (AChE), upon applying polyphenolic bioinsecticide isolated from *Streblus asper* (PBSA) at a concentration of 0.595 µg/insect ($LD_{50}$) by topical application on *Dysdercus cingulatus* Fabricius (Red cotton bug- Hemiptera, Pyrrhocoridae). The results demonstrated that the active fraction exhibited significant inhibition in activities of AChE, antioxidant enzymes and Glutathione-S-transferase (GST) and a significant increase in the lipid peroxides (MDA/ TBARS) which led to the fact that *D. cingulatus* became more susceptible to the tested PBSA. The study has provided basic information on the mechanism of action of PBSA that will be promising to develop effective alternatives to synthetic insecticides.


**KEYWORDS:** Bioinsecticide, mechanism of action, acetylcholinesterase, antioxidant enzymes, lipid peroxides

## INTRODUCTION

Cotton (*Gossipium hirsutum* L.) plant is a shrub and is widely cultivated of great economic importance in over 90 countries for its natural fiber and secondly for seeds (Chaudhry, 2010). The red cotton bug, *Dysdercus cingulatus* F. (Heteroptera, Pyrrhocoridae) is an important pest of cotton. Although synthetic chemical insecticides can control it, the side effects are enormous (Vennila *et al*., 2000). Pollution of the environment by pesticides has been increasing due to their use to manage various pests. Bioinsecticides are highly effective, safe, and ecologically acceptable (Arya *et al*., 2022). Recent emphasis is on the use of natural pesticides, which are usually of plant origin. Unlike synthetic chemical pesticides, which leave harmful residues in the aquatic environment (Aktar *et al*., 2009; Mahmood *et al*., 2016) bioinsecticides are biodegradable, specific in action (harmless to non-target organisms), and also possess the ability to counter pest resistance issues caused by synthetic pesticides (Mishra *et al*., 2020).

*Streblus asper* Lour (Family: Moraceae) is a small tree which is indigenous to tropical countries such as India, Sri Lanka, Malaysia, the Philippines and Thailand (Glasby, 1991). It is a well-known ethnomedicinal plant which is also used in Ayurveda (Singh and Singh, 1987; Singh and Ram, 1988). It finds place in the Ayurvedic Pharmacopoeia of India and an up-to-date and comprehensive review of

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*S. asper* that covers its traditional and folk medicinal uses, phytochemistry and pharmacology (Rastogi *et al*., 2006). Preliminary study has reported that polyphenolic extracts from the stem bark of S. *asper* possess insecticidal activity against the fifth instar of *D. cingulatus* (Hashim and Devi, 2003) and its partially purified polyphenolic bioinsecticide from *S. asper* (PBSA) had significant effects in the mortality of newly emerged fifth instar *D. cingulatus* (Anila and Hashim, 2022). A study on the mechanism of insecticidal activity of the most active PBSA in *D. cingulatus* by analyzing oxidative stress and inhibition of acetylcholinesterase activity upon topical application using its $LD_{50}$ concentration was taken up.

## MATERIALS AND METHODS

The red cotton bugs, were obtained from the laboratory maintained under controlled conditions (temp 28-30°C, RH 95 ± 2 % and a photoperiod of light 12 h; dark 12 h) by feeding soaked cottonseeds. Newly emerged fifth instar insects were used for the experiments. Each treatment contains three replications and fifteen insects were used for each replication.

The stem bark of the plant, *S. asper* was collected from Nagarcoil Forest (Tamil Nadu, India) and was authentically identified. Polyphenolic compounds were extracted from *S. asper* according to the procedure Hashim and Devi (2003). Two compounds were maximum insecticidal activity. The compound I with maximum insecticidal activity was identified as flavanone compound family (Anila and Hashim, 2022). PBSA was used for evaluation of mechanism of action of insecticidal activity by analyzing oxidative stress and inhibition of AChE activity upon its application on *D. cingulatus*.

Newly emerged fifth instar *D. cingulatus* (15 days after of molting) were used and insects were divided into two groups containing 12 each. Ethanol (40%) was topically applied to group I which served as control and PBSA @ 0.595 µg/insect ($LC_{50}$) dissolved in ethanol (40%) topically applied to the group II insects which served as test. After 12 and 24 hours exposure the haemolymph (40µl) was collected directly into a polystyrene tube containing few crystals of phenylthiourea by making a tiny incision in the antennae of *D. cingulatus* from each group. Hemocytes were removed from the haemolymph by centrifugation at 10000 rpm for 15 min. Separated haemolymph was used for various analyses. The whole brain was minced separately and homogenized with normal saline and centrifuged for 10 minutes at 3000 rpm and the supernatant was used for various analyses. Homogenates (as described) were performed in pools of 12 insects and every enzyme activity determination was the average of 6 independent pools of 12 insects (Daffre and Faye, 1997).

AchE activity was done by the method of Ellman *et al.* (1961). The activity was expressed as Units per milligram protein where 1 Unit = nanomoles of thiocholine liberated per minute.

Catalase (CAT) activity was measured (Maehly and Chance, 1954). The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The specific activity is expressed in terms of Units/ mg protein where 1 Unit = velocity constant per second.

The measurement of superoxide dismutase (SOD) involves generation of superoxide radical by photoreduction of riboflavin and its detection by nitrite formation from hydroxylamine hydrochloride at 543 nm (Das *et al*., 2000). SOD activity was expressed in Units/mg protein where 1 Unit = enzyme concentration required to inhibit OD at 560 nm of chromogen produced by 50 per cent in 1 minute.

Glutathione-S-transferase (GST) activity was measured spectrophotometrically by the method of Habig *et al*. (1974) using S-2,4-dinitrophenyl glutathione (CDNB) as a substrate. The principle of the method is based on measurement of the conjugation of S-2,4-dinitrophenyl glutathione (CDNB) with reduced glutathione. The formation of adduct of CDNB, S-2,4-dinitrophenyl glutathione was monitored by measuring the net increase in absorbance at 340 nm against the blank. The activity of GST was expressed in terms of l mol/min/mg protein.

The glutathione content (GSH) was determined as described by the improved method of Benke *et al*. (1974). The quantity of reduced glutathione was expressed in mg/ g protein or mg/ dl haemolymph.

Thiobarbituric acid-reacting substances (TBARS) were estimated by the method of Niehaus and Samuelsson (1968).

Protein contents of supernatant were determined after TCA (trichloro acetic acid) precipitation (Lowry, 1951) using bovine serum albumin (BSA) as the standard protein. The protein was measured at 670 nm absorbance in a spectrophotometer. Statistical significance was determined by one way Analysis of Variance (ANOVA) in SPSS 20.0 package. The data given in figures are expressed as mean ± SEM, for n = 6 experiments.

## RESULTS AND DISCUSSION

### Mechanism of insecticidal action of PBSA on AChE activity:

The control insects without any insecticide exposure exhibited an acetylcholinesterase activity of 28 units/ mg protein in haemolymph. After 12 h of PBSA exposure the enzyme activity significantly reduced to 12.95 units/mg protein and after 24 h of exposure again the activity significantly decreased to 7.89 units/mg protein. Similar pattern of inhibition was observed in enzyme activity in brain of bioinsecticide treated insects. Activity of AChE was significantly inhibited in both haemolymph and brain of red cotton bugs treated with PBSA @0.595 µg/insect ($LD_{50}$) after 12 and 24 h of topical application when compared to control insects (Table 1). The activity

Table 1. Activity of AChE in haemolymph and brain of *Dysdercus cingulatus* exposed to PBSA

| Biochemical parameters studied | After 12 hours | | | | After 24 hours | | | |
|---|---|---|---|---|---|---|---|---|
| | Haemolymph | | Brain | | Haemolymph | | Brain | |
| | Control | Test | Control | Test | Control | Test | Control | Test |
| AchE ($U^1$) | 28.16 ± 0.803 | 12.95[a] ± 0.584 | 42.36 ± 1.75 | 27.96[a] ± 1.02 | 27.53 ± 0.679 | 7.89[ab] ± 0.5511 | 42.84 ± 1.27 | 20.33[ab] ±0.694 |

[1]Unit = nanomoles of thiocholine liberated per minute per milligram protein; Values expressed as mean ± SEM, for n = 6 experiments. [a]test group is compared to control group at p = 0.05. [b]test group after 12 hours is compared to test group after 24 hours at p = 0.05

Table 2. Activity of antioxidant enzymes in haemolymph and brain of *Dysdercus cingulatus* exposed to PBSA

| Biochemical parameters studied | After 12 hours | | | | After 24 hours | | | |
|---|---|---|---|---|---|---|---|---|
| | Haemolymph | | Brain | | Haemolymph | | Brain | |
| | Control | Test | Control | Test | Control | Test | Control | Test |
| SOD ($U^2$) | 3.17 ± 0.150 | 1.85[a] ± 0.072 | 5.39 ± 0.179 | 3.17[a] ± 0.117 | 3.08 ± 0.078 | 1.16[ab] ± 0.046 | 5.46 ± 0.194 | 2.26[ab] ± 0.086 |
| CAT ($U^3$) | 16.58 ± 0.358 | 8.96[a] ± 0.142 | 32.47 ± 0.909 | 25.49[a] ± 0.610 | 16.44 ± 0.252 | 6.32[ab] ± 0.157 | 33.54 ± 0.708 | 18.67[ab] ± 0.424 |
| GST ($U^4$) | 3.46 ± 0.096 | 1.84[a] ± 0.045 | 6.31 ± 0.117 | 4.25[a] ± 0.080 | 3.22 ± 0.061 | 0.924[ab] ± 0.029 | 6.43 ± 0.104 | 3.09[ab] ± 0.070 |

Values expressed as mean ± SEM, for n = 6 experiments; [a]test group is compared to control group at p = 0.05; [b]test group after 12 hours is compared to test group after 24 hours at p = 0.05. [2] Unit = enzyme concentration required to inhibit OD at 560 nm of chromogen produced by 50 % in 1 minute. [3] Unit = velocity constant/ second; [4] Unit = m M of CDNB utilized /min/mg protein

was significantly inhibited after 24 hours of topical application when compared to 12 h exposure.

Activity of catalase and superoxide dismutase **(**SOD) was significantly inhibited in both 12 and 24 h time intervals after the topical application of PBSA @ 0.595 µg/insect ($LD_{50}$) and the inhibition was more pronounced in 24 h exposure in both haemolymph and brain. There was a significant reduction in GST activity in the haemolymph and brain of insects after the topical application of PBSA at 0.595 µg/insect ($LD_{50}$) for 12 and 24 h time intervals when compared to control insects (Table 2). The inhibition was more pronounced in the haemolymph and brain after 24 h exposure when compared to insects treated for 12 h.

Duration dependent decrease in GSH content was observed in both haemolymph and brain of PBSA at 0.595 µg/insect ($LD_{50}$) treated insects when compared to control insects. The decrease was higher in 24 h treated insects when compared to 12 h treated insects. TBARS content was significantly increased in haemolymph and brain after 12 and 24 h exposure of PBSA at 0.595 µg/insect ($LD_{50}$) treated insects when compared to control group (Table 3). There was significant increase in TBARS level in 24 h treated insects when compared to 12 h.

In the present study the polyphenolic bioinsecticide exposure have been demonstrated to reduce the activity of acetyl cholinesterase significantly in red cotton bugs. The results are in agreement with Maazoun *et al.* (2017) who reported that *Urginea maritima* bulbs extract exhibited inhibitory AChE activity in *Sitophilus oryzae* (L.). Pesticide-induced oxidative stress is the final manifestation of a multi-step pathway, resulting in an imbalance between pro-oxidant and antioxidant defense mechanisms. Concomitantly, pesticide intoxication induces a derangement of certain antioxidant mechanisms in different tissues, including alterations in antioxidant enzymes and the glutathione redox system (Banerjee *et al*., 2001). Therefore the attempt to analyze the antioxidant status and measure the activity of AChE in red cotton bugs exposed to PBSA which belongs to a flavanone family, showed a significant inhibition in the activity of AChE and antioxidant enzymes.

Reactive oxygen species (ROS), such as superoxide anions (O2) and $H_2O_2$ are produced throughout the cells during normal aerobic metabolism. The intracellular concentration of ROS is a consequence of both their production and their removal by various antioxidants. A major component of antioxidant system in mammalian cells consists of three enzymes, SOD, CAT and (glutathione peroxidase) GPX. These enzymes work in concert to detoxify $O_2$ and $H_2O_2$ in cells. It has been established that many pesticides are capable of inducing oxidative stress by overwhelming or modulating cellular drug metabolizing systems (Sule *et al*., 2022).

Oxidative stress occurs when there is an imbalance between free radical generation and antioxidant

Table 3. Concentration of reduced glutathione (GSH) and TBARS content in haemolymph and brain of *Dysdercus cingulatus* exposed to PBSA

| Biochemical parameters studied | After 12 hours | | | | After 24 hours | | | |
|---|---|---|---|---|---|---|---|---|
| | Haemolymph | | Brain | | Haemolymph | | Brain | |
| | Control | Test | Control | Test | Control | Test | Control | Test |
| MDA (TBARS) | 0.925 ± 0.047 | 1.73[a] ± 0.050 | 1.42 ± 0.053 | 2.13[a] ± 0.072 | 0.930 ± 0.042 | 2.24[ab] ± 0.075 | 1.42 ± 0.061 | 2.55[ab] ± 0.068 |
| GSH | 16.58 ± 0.592 | 9.47[a] ± 0.376 | 24.63 ± 0.581 | 12.38[a] ± 0.314 | 17.16 ± 0.551 | 6.18[ab] ± 0.330 | 25.24 ± 0.778 | 8.59[ab] ± 0.424 |

Values expressed as mean ± SEM, for n = 6 experiments; [a] test group is compared to control group at p = 0.05; [b] test group after 12 hours is compared to test group after 24 hours at p = 0.05

defenses. It often results in severe pathological consequences, such as membrane disruption, DNA damage and protein damage and cytotoxicity (Saini *et al*., 2023). The activities of the antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST) were decreased by fenitrothion incubation. The same treatment reduced the level of antioxidant glutathione (GSH). The activities of glutathione-S-transferase (GST) and gamma-glutamyl transpeptidase (gamma-GT) were more affected by fenitrothion and endosulfan, respectively, indicating oxidative stress (El-Shenawy, 2010). These support the present findings that the significant decrease in antioxidant enzymes in haemolymph and brain of bioinsecticide-treated insects when compared to control.

Recent reports showed that there were significantly reduced GSH levels in all tissues after methiocarb administration in experimental animals (Ozden *et al*., 2009) and methomyl decreased AChE, superoxide dismutase (SOD) and glutathione S-transferase (GST) activities and increased level of lipid peroxidation (LPO) (Mansour *et al*., 2009). These reports are in support with the present findings that the phenolic compound isolated from *Streblus asper* (PBSA) exhibited the insecticidal activity as evidenced by its inhibitory effect on the activities of AChE and antioxidant enzymes and significant increase in the level of lipid peroxidation.

In conclusion, polyphenolic bioinsecticide from *Streblus asper* (PBSA) has significant effects on newly emerged fifth instar *D. cingulatus*, and they caused increased mortality in a duration-dependent manner. The AChE activity and antioxidant status are impaired after the exposure of PBSA to red cotton bugs. The abnormal change in antioxidant status and acetylcholinesterase activity in cotton bugs may be the reason for the insecticidal action. The compound PBSA may therefore serve as an effective alternative to conventional insecticides in controlling red cotton bugs.

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# Bioefficacy of *Tagetes minuta* L. against *Aphis craccivora* Koch (Hemiptera, Aphididae)

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**ABSTRACT:** Efficacy of solvent fractions of *Tagetes minuta* L. (hexane, chloroform and aqueous fractions of aerial parts) was evaluated against *Aphis craccivora* Koch at five different concentrations (0.025, 0.05, 0.1, 0.15 and 0.2%) in the laboratory. At higher concentrations, all the fractions recorded significant reduction in aphid count; maximum reduction after 120h of treatment was in hexane fraction at 0.2 per cent (90.00%), followed by chloroform fraction at 0.2 per cent (86.67%) and aqueous fraction at 0.2 per cent (85.00%). The best concentrations of the three solvent fractions of *T. minuta* identified in the laboratory were evaluated against the aphid on cowpea, in a pot culture experiment. By 10th day of treatment, all the fractions at 2 per cent concentration reduced population of aphids, with the highest reduction in hexane fraction. 

**KEYWORDS:** Mexican marigold, solvent fractions, hexane fraction, aphid management

## INTRODUCTION

The cowpea aphid, *Aphis craccivora* Koch (Hemiptera, Aphididae) is an important sucking pest of leguminous crops throughout India (Jagdish *et al.*, 2011). It is a serious pest of cowpea, feeding on aerial parts of the plant and leading to significant yield loss (Veeranna and Adivappar *et al.,* 2019). It also causes indirect damage to the plant by excreting copious amount of honey dew, which leads to the development of sooty mould on the surface of leaves, thereby reducing the rate of photosynthesis; besides, it is also a vector of important viral diseases affecting the legumes (Ghosh *et al*., 2017). Synthetic insecticides are being extensively used for aphid management in vegetable crops in India. The popular chemical insecticides used against aphids include neonicotinoids like imidacloprid, acetamiprid and thiamethoxam. The excessive and indiscriminate use of synthetic pesticides for their management has, however, led to the development of resistance in the populations of aphids. *A. craccivora* has been reported to exhibit considerable degree of resistance to newer insecticides like imidacloprid (Dawood and Farghaly, 2016) and dinotefuran (Mokbel and Mohamed, 2009), among many others. Hence, there is a need for identifying alternative, environmentally benign, effective, and biodegradable pesticides with greater selectivity against the aphid pest. In this

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context, botanical pesticides are ideal alternatives for synthetic pesticides due to their rapid degradation, target specific nature and less phytotoxicity (Vasquez *et al*., 2016). *Tagetes minuta* L., commonly known as Mexican marigold is an annual herb that belongs to the family, Asteraceae. The biological activities of the plant have been documented worldwide. Insecticidal effects of *T. minuta* have been studied against several pests of field crops, stored products and public health (Perich *et al*., 1995; Shahzadi *et al*., 2010; Phoofolo *et al.,* 2013). Few studies have been conducted in India, particularly in Kerala, to evaluate the insecticidal effects of this plant; hence, a study was conducted to identify the insecticidal effects of the solvent fractions of *T. minuta* on *A. craccivora*.

## MATERIALS AND METHODS

Adults of *A. craccivora* collected from the culture maintained, in the polyhouse of Department of Agricultural Entomology, College of Agriculture, Vellanikkara, Kerala, India were used to initiate the laboratory culture. The aphids were allowed to infest cowpea seedlings grown in pro trays maintained in rearing cages in the laboratory. New seedlings were allowed to be infested periodically to maintain the culture. In order to obtain uniform aged adult aphids for the study, a few seedlings from the culture were selected and all adults were removed, retaining the nymphs. On the next day, the freshly moulted adults were collected from those seedlings and used for the study.

**Preparation of solvent fractions:** *T. minuta* plants, cultivated in the pots, in the Department of Floriculture and Landscaping, College of Agriculture, Vellanikkara, Kerala, India were used for the study. The above-ground parts of the plant were harvested at the flowering stage, shade dried and pulverized. The botanical was then extracted sequentially, based on the polarity of the solvents into three separate fractions, using hexane (non-polar), chloroform (medium polar) and water (highly polar). The aerial parts of the plant were dried and pulverized material was weighed (100g each conical flask) and extracted using hexane (3 times of volume) by placing in a rotary shaker for 48h. The solution was then filtered and the filtrate was concentrated by a rotary evaporator at 40ºC, to obtain the hexane fraction. The residue obtained during filtration was re-extracted sequentially using chloroform, followed by water, following the same procedure as hexane extraction, to obtain chloroform fraction and aqueous fraction, respectively (Auamcharoen and Chandrapatya, 2015). The three solvent fractions were used for bioefficacy studies against *A. craccivora.*

**Laboratory bioassay:** The hexane, chloroform and aqueous fractions of *T. minuta* were evaluated at five different concentrations (0.025, 0.05, 0.1, 0.15 and 0.2 %) for efficacy against *A. craccivora*. The treatment concentrations were prepared by dissolving the required quantity of each fraction in distilled water along with the emulsifier, Triton X-100 (20µl $50ml^{-1}$). Cowpea seeds were sown in individual paper cups (one seed per cup) filled with potting mixture (soil and vermicompost in 1:1 ratio) and the seeds were allowed to sprout and grow. At four leaf stage, adult aphids from the laboratory culture were released using a camel hair brush at the rate of 20 per plant and allowed to settle. The seedlings were then sprayed with appropriate treatments using a hand atomizer (3ml per seedling). Three replications were maintained for each concentration of the fractions. Seedlings sprayed with water plus emulsifier, served as control. An absolute control was also maintained without any treatment. The experiment was laid out in Completely Randomized Design (CRD) with 17 treatments and three replications. Nymphs produced viviparously if any, were removed from the experimental seedlings periodically, to avoid any experimental error due to population build up. Number of adult aphids on the plants was recorded at 24, 48, 72, 96 and 120h of spraying and reduction in aphid count was calculated. Data on reduction of aphid was subjected to analysis of variance using the software, GRAPES 1.0.0, developed by Kerala Agricultural University.

**Evaluation of fraction in pot culture:** A pot culture experiment was carried out to evaluate the efficacy of the most effective concentration of each fraction of *T. minuta* against *A. craccivora* in cowpea, during July - September, 2022. The best

concentration each of hexane (0.2%), chloroform (0.2%) and aqueous (0.2%) fractions were selected for evaluation based on their efficacy in the laboratory study against the adult aphids. The efficacy of the selected fractions was compared with those of neem oil emulsion (2%), azadirachtin (1% EC, 3ml $L^{-1}$) and horticultural mineral oil (2%). An untreated control was also maintained. The experiment was laid out in CRD with seven treatments, each replicated thrice with eight plants per replication.

The seeds of cowpea variety - Bhagyalakshmi (bush type) were sown in individual polybag (35cm x 20cm x 20cm) filled with potting mixture (soil, coir pith compost and cow dung in 2:1:1 ratio). All cultural practices were carried out as per Package of Practices Recommendations, KAU (2016). Aphids were allowed to infest the 20-day-old cowpea plants by keeping aphid infested cowpea seedlings in paper cups, at the base of each plant in polybag. Treatments were imposed 20 days after the release of aphids, using a hand sprayer. For recording the aphid population in treatments, three plants were selected randomly from each treatment replication. Number of aphids (both nymphs and adults) was recorded from three shoot bits of 5cm length per plant, excised from three tender shoots (Bindu, 1997). Pre-treatment count one day prior to treatment and post treatment counts of aphids at 1, 3, 7 and 10 days after treatment (DAT) application were counted by dislodging the aphids from each shoot bit, on to a white paper.

The data on mean number of aphids (per 5cm shoot length) before and after treatments were subjected to analysis of covariance (ANCOVA) using the software, GRAPES 1.0.0. In order to accommodate the variations in pre-count, the transformed data were analysed by taking population counts prior to the first application as covariate and ANCOVA was done for observations at 1, 3, 7 and 10 DAT. The result obtained was subjected to LSD (Least Significance Difference Test). The mean per cent reduction in population was also worked out 10 DAT.

**Qualitative phytochemical analysis:** Qualitative analysis of hexane (nonpolar) and chloroform (medium polar) fractions of *T. minuta*, was performed to determine the predominant phytochemical constituents in the fractions. However, aqueous (polar) fraction was not analysed as ample literature on the constitution of polar fractions of *T. minuta* is available. The hexane and chloroform fractions were subjected to GC-MS/MS analysis at Sophisticated Analytical Instrumentation Facility (SAIF), IIT, Mumbai and the major compounds present in the fractions were recorded.

## RESULTS AND DISCUSION

**Laboratory evaluation of solvent fractions:** There was a sudden decline in aphid count on treated seedlings up to 48h of treatment, after which the population reduction was less pronounced. All three solvent fractions recorded the maximum reduction in aphid count at the highest concentration (0.2%) tested. Within 24h of treatment the hexane fraction (@0.2%) recorded 61.67 per cent reduction, followed by chloroform (60%) and aqueous fractions (58.33%), which were on par with each other. At lower concentration (0.15%), hexane fraction and aqueous fraction also reduced aphid count (60 and 50% respectively) and were on par with the above treatments. After 48h, hexane fraction (@0.2%) resulted in as high as 83.33 per cent reduction. Chloroform fraction (0.2%) also showed significant reduction (78.33%), on par the hexane fraction. Other concentrations of solvent fractions also showed reduction in aphid count at 48h. From 48 to 120h of treatment, the reduction in aphid count was less prominent in all the treatments. After 120 h of treatment, there were significantly higher reduction in aphid count in hexane fraction (@0.2%), hexane fraction (@0.15%), chloroform fraction (@0.2%), chloroform fraction (@0.2%) and aqueous fraction (@0.2%) (recording 90.00, 86.67, 86.67, 85.00 and 85.00% respectively) and were on par with one another (Table 1).

**Evaluation of fractions in pot culture:** All the treatments reduced the aphid population considerably 10 DAT. Among the fractions, hexane fraction recorded the lowest population (11.18 aphids per 5cm of shoot) resulting in 78.95 per cent reduction in aphid population. The hexane fraction

Table 1. Laboratory evaluation on the bioefficacy of *Tagetes minuta* solvent fractions against *Aphis craccivora*

| Treatment | Reduction in aphid count (%) – hours after treatment | | | | |
|---|---|---|---|---|---|
| | 24h | 48h | 72h | 96h | 120h |
| Hexane fraction 0.025 % | 23.33$^{fg}$ (28.67) | 40.00$^{hi}$ (39.21) | 53.33$^{gh}$ (46.92) | 56.67$^{gh}$ (48.84) | 56.67$^{f}$ (48.84) |
| Hexane fraction 0.05 % | 40.00$^{cde}$ (39.21) | 53.33$^{fg}$ (46.94) | 60.00$^{fg}$ (50.85) | 65.00$^{fg}$ (53.76) | 65.00$^{de}$ (53.76) |
| Hexane fraction 0.1% | 43.33$^{cd}$ (41.13) | 63.33$^{e}$ (52.78) | 68.33$^{def}$ (55.82) | 75.00$^{de}$ (60.07) | 76.67$^{bc}$ (61.15) |
| Hexane fraction 0.15 % | 60.00$^{ab}$ (50.79) | 75.00$^{b}$ (60.07) | 76.67$^{bcd}$ (61.15) | 80.00$^{cd}$ (63.55) | 86.67$^{a}$ (68.66) |
| Hexane fraction 0.2 % | 61.67$^{a}$ (51.81) | 83.33$^{a}$ (65.95) | 86.67$^{a}$ (68.66) | 88.33$^{a}$ (70.11) | 90.00$^{a}$ (71.57) |
| Chloroform fraction 0.025 % | 30.00$^{ef}$ (33.16) | 48.33$^{gh}$ (44.04) | 55.00$^{gh}$ (47.88) | 56.67$^{gh}$ (48.84) | 58.33$^{ef}$ (49.80) |
| Chloroform fraction 0.05 % | 36.67$^{de}$ (37.20) | 65.00$^{de}$ (53.76) | 70.00$^{de}$ (56.84) | 71.67$^{ef}$ (57.86) | 73.33$^{bc}$ (59.00) |
| Chloroform fraction 0.1 % | 38.33$^{cde}$ (38.19) | 66.67$^{cde}$ (54.75) | 71.67$^{cde}$ (57.86) | 75.00$^{de}$ (60.00) | 76.67$^{bc}$ (61.15) |
| Chloroform fraction 0.15 % | 48.33$^{bcd}$ (44.01) | 73.33$^{bc}$ (58.93) | 80.00$^{abc}$ (63.55) | 81.67$^{bcd}$ (64.81) | 85.00$^{a}$ (67.40) |
| Chloroform fraction 0.2 % | 60.00$^{ab}$ (50.79) | 78.33$^{ab}$ (62.29) | 85.00$^{a}$ (67.40) | 86.67$^{ab}$ (68.66) | 86.67$^{a}$ (68.66) |
| Aqueous fraction 0.025% | 15.00$^{gh}$ (22.29) | 36.67$^{I}$ (37.26) | 45.00$^{h}$ (42.12) | 46.67$^{I}$ (43.09) | 48.33$^{g}$ (44.04) |
| Aqueous fraction 0.05% | 28.33$^{ef}$ (32.14) | 45.00$^{ghi}$ (42.12) | 53.33$^{gh}$ (46.92) | 55.00$^{hi}$ (47.91) | 58.33$^{ef}$ (49.83) |
| Aqueous fraction 0.1% | 38.33$^{cde}$ (38.22) | 61.67$^{ef}$ (51.76) | 65.00$^{ef}$ (53.76) | 70.00$^{ef}$ (56.79) | 70.00$^{cd}$ (56.79) |
| Aqueous fraction 0.15% | 50.00$^{abc}$ (45.00) | 63.33$^{e}$ (52.74) | 71.67$^{cde}$ (57.86) | 75.00$^{de}$ (60.07) | 78.33$^{b}$ (62.29) |
| Aqueous fraction 0.2 % | 58.33$^{ab}$ (49.80) | 71.67 $^{bcd}$ (57.86) | 81.67$^{ab}$ (65.00) | 83.33$^{abc}$ (66.15) | 85.00$^{a}$ (67.40) |
| Control (Water + emulsifier) | 13.33$^{h}$ (21.34) | 13.33$^{j}$ (21.34) | 16.67$^{I}$ (24.05) | 18.33$^{j}$ (25.31) | 18.33$^{h}$ (25.31) |
| Absolute control | 1.67$^{i}$ (4.31) | 10.00$^{j}$ (18.43) | 11.67$^{I}$ (19.89) | 13.33$^{j}$ (21.34) | 13.33$^{h}$ (21.34) |
| LSD (0.05) | 7.329 | 4.881 | 5.789 | 4.974 | 4.568 |

Figures in parentheses are arc sine transformed values; Means followed by common letter(s) do not significantly differ at P=0.05%

was on par with the neem oil emulsion (7.57 aphids/ 5cm shoot) that reduced aphid population by 87.59 per cent. The chloroform and aqueous fractions were on par with each other, reducing aphid population by 72.43 and 68.20 per cent, respectively. Aqueous fraction recorded aphid population (23.11) on par with azadirachtin (25.74), whereas, chloroform fraction was superior to azadirachtin recording a lower population of 18.41 aphids per 5 cm of shoot (Table 2).

**Phytochemical constituents in solvent fractions:** Qualitative phytochemical analysis of the bio-active fractions *viz.*, hexane and chloroform fractions GC-MS/MS identified the presence of 4á-phorbol 12,13-didecanoate, 4-o-methylphorbol 12, 13- didecanoate and milbemycin b in hexane fraction and 4-o-methylphorbol 12, 13- didecanoate and milbemycin b in the chloroform fraction.

In the laboratory, on treatment application, the aphids moved away from the treated seedlings, suggesting a strong repellent and/or antifeedant action by the solvent fractions of *T. minuta*. In the pot culture study, the solvent fractions could significantly reduce the aphid population on cowpea. The efficacy of the hexane fraction of *T. minuta* was found comparable to neem oil while that of chloroform and aqueous fractions was comparable to azadirachtin 1 EC, which are widely recommended in the management of aphids in various crops (Sarvaiya *et al*., 2018).

Volatile constituents present in several botanicals are known to trigger sudden repellent action in the aphids (Dardouri *et al.*, 2019). Volatile compounds such as D-limonene, dihydro-tagetone, (E)-tagetone, (Z)-tagetone, (Z)-beta-ocimene and allo-ocimene were reported to be present in *T. minuta* and are known to have repellent properties against insects (Kimutai *et al*., 2015). Polar fractions of *T. minuta*, extracted using polar solvents such as methanol and water were reported to be composed

Table 2. Effect of *Tagetes minuta* solvent fractions on *Aphis craccivora* in cowpea pot culture

| Treatment | Mean live aphids/ 5cm shoot length at - DAT | | | | | Reduction (%) |
|---|---|---|---|---|---|---|
| | PTC* | 1 | 3 | 7 | 10 | |
| Hexane fraction 0.2 % | 53.11 (7.28) | 20.37$^{c}$ (4.51) | 15.29$^{d}$ (3.91) | 12.59$^{c}$ (3.54) | 11.18$^{d}$ (3.33) | 78.95 |
| Chloroform fraction 0.2% | 66.78 (8.17) | 27.33$^{b}$ (5.22) | 23.33$^{bc}$ (4.81) | 19.69$^{b}$ (4.39) | 18.41$^{c}$ (4.27) | 72.43 |
| Aqueous fraction 0.2% | 72.67 (8.52) | 30.04$^{b}$ (5.48) | 20.74$^{c}$ (4.55) | 18.63$^{b}$ (4.31) | 23.11$^{bc}$ (4.81) | 68.20 |
| Neem oil emulsion 2% | 61.18 (7.82) | 11.89$^{d}$ (3.45) | 9.96$^{e}$ (3.16) | 7.93$^{d}$ (2.81) | 7.59$^{de}$ (2.75) | 87.59 |
| Azadirachtin 1% EC (3 ml L $^{-1}$) | 80.37 (8.96) | 29.00$^{b}$ (5.38) | 25.85$^{b}$ (5.08) | 24.63$^{b}$ (4.95) | 25.74$^{b}$ (5.07) | 67.97 |
| Horticultural mineral oil 2% | 33.93 (5.82) | 8.45$^{e}$ (2.90) | 6.48$^{f}$ (2.55) | 6.38$^{d}$ (2.52) | 5.59$^{e}$ (2.36) | 83.52 |
| Untreated control | 43.74 (6.61) | 50.41$^{a}$ (7.09) | 44.36$^{a}$ (6.66) | 51.22$^{a}$ (7.15) | 61.78$^{a}$ (7.85) | – |
| LSD (0.05) | NS | 0.413 | 0.478 | 0.715 | 0.590 | – |

*PTC=Pre-treatment count; #Reduction at10 DAT (%); Figures in parentheses are square root transformed values; Means followed by common letter (s) do not significantly differ at P=0.05 %

of various carbohydrates, proteins, phenols, flavonoids, terpenoids and alkaloids (Rikisahedew, 2018). The terpenoid, D-limonene present in the polar fraction of *T. minuta* is known to possess significant insecticidal properties (Karr and Coats, 1987).

Qualitative phytochemical analysis of hexane and chloroform fractions in the present study showed phorbol esters as predominant compounds. The antifeedant properties of the phorbol esters against some insect pests have already been documented. Phorbol esters extracted from *Jatropha* oil were evaluated for insecticidal activity against the third instar larvae of *Spodoptera frugiperda* (JE Smith). At highest concentration of the phorbol esters (0.25 mg $ml^{-1}$ w/v), food consumption and relative growth rate of *S. frugiperda* larvae reduced (by 33 and 42% respectively), apart from exhibiting contact toxicity (Devappa *et al*., 2012). Phorbol esters are also known to affect signal transduction pathways by acting on the biological membranes (Goel *et al.*, 2007). Among the two phorbol esters identified, the role of 4á-phorbol 12, 13-didecanoate as a prominent TRPV (Transient receptor potential cation channel) agonist has been widely documented (Alexander *et al*., 2012). Similarly, milbemycin b, identified in both hexane and chloroform fractions, is a GABA gated chloride channel agonist (Ozoe, 2012), like the avermectin group of insecticides. The insecticidal activities of this compound have been widely documented against several insects (Bobade, 2019).

There are also reports on the detrimental effects of volatile compounds present in *T. minuta viz.,* â-caryophyllene, limonene and (Z) ocimene on the reproduction of aphids. Tomova *et al.* (2005) reported up to 100 per cent reduction in reproduction in three species of aphids, *viz., Acyrthosiphon pisum*, *Myzus persicae* and *Aulacorthum solani*, on exposure to these compounds. The efficacy of the *T. minuta* fractions against *A. craccivora* in the present study could be the combined effect of repellency, antifeedancy, and neurotoxicity and reproduction inhibition. Though insecticidal effects of *T. minuta* against different species of aphids have been documented from different parts of the world, most of the studies evaluated the crude aqueous extracts of the botanical in the field. Limited studies were conducted to evaluate the solvent fractions of *T. minuta.* When Ali *et al*. (2019) evaluated the bio-efficacy of aqueous leaf extracts of *T. minuta* along with three other botanicals *viz., Calotropis procera, Argemone mexicana* and *Azadirachta indica* against the mustard aphid, *Lipaphis erysimi* on Indian mustard, *Brassica juncea*, at higher concentration (1: 2.5 g $ml^{-1}$), *T. minuta* reduced *L. erysimi* population by 96.38 per cent. Kora and Teshome (2016) evaluated the aqueous extracts of chilli, garlic, ginger and *T. minuta* for their insecticidal property against green pea aphid, *Acrythosiphon pisum* in the field. The aphid population was reduced to zero in the plants treated with *T. minuta*, chilli and ginger. The above studies show that *T. minuta* extracts can reduce aphid population build up considerably, in the field. Phoofolo *et al*. (2013) had evaluated the aphidicidal activities of the crude extracts *T. minuta* against the cabbage aphid, *Brevicoryne brassicae*. A comparison was made on lethal and sub-lethal effects of the crude extracts from acetone, methanol, water and a mixture of acetone/methanol/water (7:7:1 v:v). The mixture produced the most toxic extract, followed by methanol and water; whereas acetone extract was the least toxic. The study also demonstrated that the crude extract of *T. minuta* obtained using water as a solvent is as effective as crude extracts from organic solvent systems in terms of efficacy against cabbage aphids. This observation corresponds to the results of the present study where all the three solvent fractions of *T. minuta* (hexane, chloroform and water) were effective in reducing the aphid population.

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# Exploration of natural enemy fauna of aphids and associated ant species from eastern dry zone of Karnataka, India

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**ABSTRACT:** Purposive surveys were conducted at regular intervals in different districts of eastern dry zone of Karnataka (Zone 5) during the year 2021-22, to document the natural enemy fauna and the ant species associated with aphids. Out of 34 aphid species recorded, 16 species of aphids were preyed on by 14 species of predators belonging to Coccinellidae, Syrphidae, Hemerobiidae and Chamaemyiidae and seven species of aphids were found parasitized by eight species of parasitoids belonging to Braconidae. Predators belonging to Coccinellidae and Syrphidae were recorded more abundantly with six species in each family, preying on 12 and 10 aphid species, respectively. Among the aphid parasitoids, *Aphidius* spp. was the more abundant taxa. Eleven species of ants belonging to Formicinae, Myrmicinae and Dolichoderinae were found associated with 17 aphid species. Ants belonging to the genus *Camponotus* were found to be more abundant and associated with 10 species of aphids. A comprehensive list of predators, parasitoids and ants associated with different aphid species was put together during this study. 

**KEY WORDS:** Purposive surveys, abundance, predators, parasitoids, aphidocolous ants

## INTRODUCTION

Aphids (Hemiptera, Aphididae) are small soft bodied sap sucking insects. More than 450 species of aphids are found to be associated with different crop plants (Blackman and Eastop, 2000) of which about 100 species are of economic importance. Chemical insecticides have been used regularly for the management of aphid pests but not without risk of resurgence, destruction of natural enemies, development of resistance, phytotoxic effects, environmental pollution and residual toxicity. Changing scenario of modern sustainable agriculture emphasizes the need for biological control for effective management of aphids. Aphids are good candidates for biological control (Joshi *et al*., 2010) as they serve as a consistent and abundant food source for many natural enemies (Singh and Singh, 2016). Aphids have a mutualistic relationship with the ants which is by way of the aphids providing rich supply of food for ants in the form of honey dew and in return receiving

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protection from their natural enemies. The risk to aphids of death caused by predators can be decreased through recruiting more ants by producing more attractive honeydew (Stadler and Dixon, 2005). Presence of ants also accelerates growth of aphids and establishment of aphid colony (Saha *et al*., 2018). Ants that attend aphids belong largely to the most evolved subfamilies Myrmicinae, Formicinae and Dolichoderinae (Tizado *et al*., 1993). Noteworthy contributions on the natural enemies of aphids in India were made by Rao (1969), Raychaudhuri (1978), Ghorpade (1981), Ghosh and Raychaudhuri (1982), Stary and Ghosh (1983), Agarwala and Ghosh (1988), Singh *et al*. (1999), Joshi (2005), Dey and Akthar (2007), Akthar *et al*. (2011), Chaudhary and Singh (2012), Joshi and Sangma (2015), Bhat and Bhagat (2017), Khan *et al*. (2017), Bhat *et al*. (2020), Kale *et al*. (2020) and Maji *et al*. (2023). In India, aphid-ant association has been mainly dealt with Gadiyappanavar (1970), Roy and Behura (1980), Kurl and Misra (1980), Datta *et al*. (1982, 1983), Devi and Singh (1986), Verghese and Tandon (1987), Devi *et al*. (1987), Devi *et al*. (2001), Bisht *et al*. (2002), Joshi (2005), Kataria and Kumar (2013) and Rakshan and Ahmad (2015). The present investigation was carried out with an aim to record the natural enemies and ant species associated with aphids from eastern dry zone of Karnataka (Zone 5).

## MATERIALS AND METHODS

In order to record the various natural enemies associated with different species of aphids, purposive surveys were conducted at regular intervals (15 days) in six districts of eastern dry zone of Karnataka *viz*., Bengaluru Urban (13.0801° N; 77.5406° E), Bengaluru Rural (13.3535° N; 77.5406° E), Kolar (13.1320°N; 78.1783° E), Chikkaballapura (13.3354°N; 78.0824°E), Tumkur (13.2818° N; 77.1860°E) and Ramanagara (12.9576°N; 77.2261°E) during 2021-22. Field collection of aphid infested plant parts and associated aphid predators was carried out. The aphids collected were preserved in small plastic vials containing ethyl alcohol (70%), properly furnished with labels and the slides were prepared in accordance with the method suggested by Eastop and van Emden (1972). Identification of aphids up to species level was carried out using the keys available from Aphids on the world's plants: an online identification and information guide and on confirmation of the identity with the specialist. Immature stages of predators associated with aphids were collected in small plastic containers and brought to the laboratory. These were reared to adult stages in rearing containers, providing respective host aphids as food (Joshi *et al*., 1997). To record the parasitoid species associated with aphids, a part of the collection of aphid infested plant material and also colony with mummified aphids were brought to the laboratory (Joshi, 2005). Emerging adult parasitoids and predators were collected and processed for study. Identification of the predators and parasitoids were carried out with the help of specialists in the respective fields. Ants associated with different species of aphids were collected directly from the field and preserved in small vials containing alcohol (70%) for identification.

## RESULTS AND DISCUSSION

During the course of investigation, out of 34 aphid species recorded, 16 species of aphids were preyed on by 14 species of predators belonging to four families and seven species of aphids were found parasitized by eight species of parasitoids belonging to Braconidae. The aphidophagous predators belonged to Coccinellidae, Syrphidae, Hemerobiidae and Chamaemyiidae. Six species each of aphidophagous coccinellid beetles and syrphids were recorded preying on 12 and 10 aphid species, respectively. Members of Hemerobiidae were recorded feeding on six aphid species and *Leucopis* sp. belonging to Chamaemyiidae was recorded preying on three aphid species (Table 1). Out of the eight aphid parasitoids, *Aphidius* spp. was recorded parasitizing five aphid species. Other parasitoids recorded include *Binodoxys* sp., *Lysiphlebus* sp. and *Trioxys* sp. which were recorded feeding on single aphid species each (Table 2). Out of 34 species of aphids reported, 17 species of aphids were found associated with 11 species of ants (Table 3). Three subfamilies of Formicidae were reported. Subfamily Formicinae was found more abundant with five species,

Table 1. Predatory species associated with different species of aphids in eastern dry zone of Karnataka during 2021-22

| Predator | Aphid | Host plants |
|---|---|---|
| *Cheilomenes sexmaculata* (F.) Coccinellidae | *Aphis craccivora* Koch | *Cajanus cajan* (L.); *Cyamopsis tetragonoloba* (L.); *Dolichos lablab* L.; *Vigna unguiculata* (L.) |
| | *A. odinae* (van der Goot) | *Anacardium occidentale* L. |
| | *Brevicoryne brassicae* (Linnaeus) | *Brassica oleraceae* L. var. *capitata* |
| | *Hysteroneura setariae* (Thomas) | *Melinis repens* (Willd.) Zizka |
| | *Hyadaphis coriandri* Das | *Anethum graveolens* L. |
| | *Macrosiphum rosae* (Linnaeus) | *Rosa* sp. |
| | *Myzus persicae* (Sulzer) | *Br. juncea* L. |
| | *Rhopalosiphum maidis* (Fitch) | *Zea mays* L. |
| | *Schoutedenia emblica* (Patel and Kulkarni) | *Phyllanthus emblica* L. |
| *Coccinella transversalis* Fabricius Coccinellidae | *A. craccivora* | *Arachis hypogea* Willd |
| | *Br. brassicae* | *Br. oleraceae* L. var. *capitata* |
| | *M. persicae* | *Br. juncea* |
| | *R. maidis* | *Z. mays* |
| *Propylea dissecta* (Mulsant) Coccinellidae | *A. craccivora* | *Arachis hypogea C. cajan* |
| *Pseudaspidimerus* sp. Coccinellidae | *A. citricida* (Kirkaldy) | *Artocarpus heterophyllus* Lamk. |
| | *A. odinae* | *Garcinia indica* Choisy |
| *Scymnus nubilus* Mulsant Coccinellidae | *R. maidis* | *Z. mays* |
| *S. latemaculatus* Motschulsky Coccinellidae | *A. craccivora* | *Gliricidia maculate* (Jacq.) |
| | *A. gossypii* Glover | *Hibiscus rosa sinensis* L. |
| | *A. nerii* Boyer de Fonscolombe | *Calotropis gigantea* (L.) |
| *Asarkina belli* Ghorpade Syrphidae | *A. gossypii* | *H. rosa sinensis*. |
| *Betasyrphus* sp. Syrphidae | *A. craccivora* | *C. cajan* |
| | *A. nerii* | *C. gigantea* |
| | *Lipaphis pseudobrassicae* (Davis) | *Br. juncea* |
| | *M. persicae* | *Br. juncea* |
| *Dideopsis aegrota* (F.) Syrphidae | *A. odinae* | *Anacardium occidentale* L. |
| *Episyrphus viridaureus* (Wiedemann); Syrphidae | *Macrosiphum rosae* (Linnaeus) | *Rosa* sp. |
| *Ischiodon scutellaris* (Fabricius) Syrphidae | *A. craccivora* | *C. cajan* |
| | *A. gossypii* | *Cucumis sativa* L.; *Chromolaena odorata* (L.) |
| | *A. citricida* | *Citrus* sp. |
| | *Hyperomyzus carduellinus* (Kirkaldy) | *Sonchus* sp. |

| | | |
|---|---|---|
| *Serratoparagus serratus* (Fabricius) Syrphidae | *A. craccivora* | *C. cajan*; *Cyamopsis tetragonoloba* (L.); *G. maculata* |
| | *A. citricida* | *Citrus* sp. |
| | *R. maidis* | *Z. mays* |
| Unidentified Hemerobiidae | *A. craccivora* | *Vigna unguiculata* (L.) |
| | *A. odinae* | *Aralia* sp. |
| | *A. gossypii* | *Lantana camara* L. |
| | *Macrosiphum euphorbiae* (Thomas) | *Rosa* sp. |
| | *R. maidis* | *Zea mays* L. |
| | *Uroleucon compositae* (Theobald) | *Phyllocephalum scabridum* (DC.) |
| *Leucopis* sp. Chamaemyiidae | *A. gossypii* | *H. rosachinensis* |
| | *Hysteroneura setariae* (Thomas) | *Melinis repens* (Willd.) Zizka |
| | *R. maidis* | *Z. mays* |

Table 2. Parasitoid species [Braconidae, Aphidiinae] associated with different species of aphids in the eastern dry zone of Karnataka during 2021-22

| Parasitoid | Host aphid | Host plants |
|---|---|---|
| *Aphidius matricariae* Haliday | *Aulacorthum solani* (Kaltenbach) | *Brugmansia suaveolens* (Humb. & Bonpl. Ex Willd.) |
| *Aphidius* sp. | *Macrosiphum rosae* (Linnaeus) | *Rosa* sp. |
| *Aphidius* sp. | *Myzus persicae* (Sulzer) | *Brassica juncea* L. |
| *Aphidius* sp. | *Macrosiphoniella sanborni* (Gillette) | *Chrysanthemum indicum* L. |
| *Aphidius* sp. | *Lipaphis pseudobrassicae* (Davis) | *Brassica oleraceae* L. var *capitate* |
| *Binodoxys* sp. | *A. gossypii* Glover | *Duranta* sp. |
| *Lysiphlebus* sp. | *A. craccivora* Koch | *Arachis hypogea* Willd. |
| *Trioxys* sp. | *A. craccivora* | *Cyamopsis tetragonoloba* (L.) |

followed by Myrmicinae and Dolichoderinae with three species each.

Among the coccinellid predators, more common was *Cheilomenes sexmaculata* (Fabricius), which was found preying on nine species of aphids followed by *Coccinella transversalis* Fabricius which preyed on four aphid species. Pervez (2004) provided a catalogue of predaceous coccinellids of India and its prey, which also gives an account of *Ch. sexmaculata* and *Co. transversalis* as predators. In the study conducted by Megha *et al.* (2015) on coccinellids in different crops at Dharwad region of Karnataka, *Ch. sexmaculata* was the dominant species, consistent with the present findings. The catalogue of predaceous coccinellids of India gives the account of *Propylea dissecta* (Mulsant) feeding on *Aphis craccivora* Koch (Pervez, 2004). Agarwala and Ghosh (1988) provided prey records of aphidophagous coccinellids in India, which gives an early record of *Pseudaspidimerus* sp. preying on the *Aphis citricida* (Kirkaldy) and *A. odinae* (van der Goot). Megha *et al.* (2015) recorded *Scymnus nubilus* Mulsant feeding on *Rhopalosiphum maidis* (Fitch) from Dharwad region of Karnataka and the record of *S. latemaculatus* Motschulsky preying on *A. craccivora*, *A. gossypii* Glover and *A. nerii* Boyer

Table 3. List of aphidocolous ant species recorded from eastern dry zone of Karnataka during 2021-22

| Ant species | Associated aphid species | Host plants |
|---|---|---|
| Subfamily - Formicinae | | |
| *Anoplolepis gracilipes* (Smith) | *Aphis gossypii* Glover | *Gardenia resinifera* Roth |
| | *A. nerii* | *Calotropis gigantea* (L.) |
| | *A. odinae* | *Mussaenda erythrophylla* Lam. |
| | *Pseudoregma bambucicola* (Takahashp) | *Bambusa vulgaris* Schrad. ex J. C. Wendl. |
| | *Rhopalosiphum maidis* | *Zea mays* L. |
| *Camponotus* sp. | *A. craccivora* | *Cajanus cajan* (L.)<br>*Cyamopsis tetragonoloba* (L.)<br>*Gliricidia maculate* (Jacq.) |
| | *A. fabae* | *Solanum nigrum* L. |
| | *A. gossypii* | *Ruellia britonniana* Leonard<br>*Catharanthus rosea* (L.) |
| | *A. spiraecola* Patch | *Bidens Pilosa* L. |
| | *A. citricida* (Kirkaldy) | *Artocarpus heterophyllus* Lamk.<br>*Citrus aurantifolia* Christm., *Citrus* sp. |
| | *A. odinae* (van der Goot) | *Hamelia patens* Jacq. *Mussaenda erythrophylla* Lam. |
| | *Cinara tujafilina* (del Guercio) | *Thuja chinensis* Borders and Gausen |
| | *Hysteroneura setariae* (Thomas) | *Eleusine corocana* (L.) *Melinis repens* (Willd.) Zizka |
| | *Rhopalosiphum maidis* (Fitch) | *Zea mays* L. |
| | *Schoutdenia emblica* (Patel "and Kulkarni) | *Phyllanthus emblica* L. |
| *Crematogaster* sp. | *A. craccivora* | *Solanum torvum* Sw. |
| | *A. gossypii* | *Hibiscus rosa sinensis* L.<br>*Lantana camara* L. |
| *Oecophylla smaragdina* Smith | *A. gossypii* | *Chromolaena odorata* (L.) |
| | *A. citricida* (Kirkaldy) | *Citrus* sp. |
| | *A. odinae* (van der Goot) | *Anacardium occidentale* L. |
| | *Schoutdenia emblica* (Patel and Kulkarni) | *Phyllanthus emblica* L. |
| *Paratrechina* sp. | *A. gossypii* | *Ocimum sanctum* L. |
| Subfamily - Myrmicinae | | |
| *Lophomyrmex* sp. | *A. gossypii* | *Chromolaena odorata* (L.) |
| *Myrmicaria brunnea* (Saunders) | *A. craccivora* | *Cyamopsis tetragonoloba* (L.)<br>*Dolichos lablab* L.<br>*Moringa oleifera* Lam. |
| | *A. gossypii* | *Parthenium hysterophorus* L.<br>*Tecoma stans* (L.) |
| | *A. odinae* | *Mussaenda erythrophylla* Lam.<br>*Tagetes erecta* L. |
| | *H. setariae* | *Melinis repens* (Willd.) Zizka |

| | | |
|---|---|---|
| *Solenopsis* sp. | *A. craccivora* | *Arachis hypogea*<br>*Vigna radiata* (L.) |
| | *A. gossypii* | *Abelmoschus esculentus* Moench |
| | *A.odinae* | *Anacardium occidentale* |
| | *H. setariae* | *Eleusine indica* (L.) |
| | *Uroleucon compositae* (Theobald) | *Guizotia abyssnicia* (L.f.) Cass. |
| Subfamily - Dolichoderinae | | |
| *Tapinoma melanocephalum* (F) | *Aphis spiraecola* Patch | *Chromolaena odorata* (L.) |
| *Technomyrmex albipes* (Smith) | *A. odinae* | *Pentas* sp. |
| *Technomyrmex* sp. | *A. craccivora* | *Arachis hypogea Cordyline* sp. |
| | *A. odinae* | *Aralia* sp. |
| | *Cerataphis brasiliensis* (Hempel) | *Areca catechu* L. |
| | *Pentalonia caladii* Boyer de Fonscolombe | *Alpinia zerumbet* (Pers.) |
| | *Pentalonia nigronervosa* Coquerel | *Musa* sp. |
| | *Melanaphis sacchari* (Zehntner) | *Saccharum officinarum* L. |
| | *Schoutdenia emblica* (Patel and Kulkarni) | *Phyllanthus emblica* L. |

de Fonscolombe have also been made earlier (Chaudhary and Singh, 2012), similar to the findings of this study.

Among the syrphids recorded during the present study, *Ischiodon scutellaris* (Fabricius) and *Betasyrphus* sp. were found in abundance preying on four aphid species each. The syrphid, *Serratoparagus serratus* (Fabricius) was recorded feeding on three species of aphids. *Asarkina belli* Ghorpade, *Dideopsis aegrota* (Fabricius) and *Episyrphus viridaureus* (Wiedemann) were also recorded, each feeding on a single species. Similar reports of these aphid-syrphid associations were also given by Ghorpade (1981). Hemerobiidae are major biocontrol agents that are used against aphids in several parts of the world. Members of Chamaemyiidae such as *Leucopis glyphinivora* Tanas. are potential biocontrol agents against aphids (Singh and Singh, 2016).

During the investigation, eight aphid parasitoid species belonging to Braconidae were recorded. Joshi (2005) reported *Aphidius* spp. and *Binodoxys* sp. parasitizing the aphids analogous to the records made during the present study. Similarly, the catalogue of aphid, parasitoids (Braconidae, Aphidiinae) from India also provides an account of *Aphidius* sp., *Lysiphlebus* sp. and *Trioxys* sp. parasitizing the aphids (Akthar *et al*., 2011).

In the present study the *Camponotus* ants were more abundant, associated with ten species of aphids. Seven species of aphids were attended by *Technomyrmex* sp., *Anoplolepis gracilipes* (Smith) and *Solenopsis* sp. were found associated with five species of aphids. *Oecophylla smaragdina* Smith and *Myrmicaria brunnea* (Saunders) attended four species of aphids. *Crematogaster* sp. was found associated with two species of aphids. *Paratrechina* sp., *Lophomyrmex* sp., *Tapinoma melanocephalum* (Fabricius) and *Technomyrmex albipes* (Smith) attended one species of aphids each. Rohini (2017) also recorded 26 aphid species from Chikkamagaluru district and reported 11 species of ants associated with 12 species of aphids, where the genus *Camponotus* was encountered more commonly tending six species of aphids, *Solenopsis* sp. attended three species of aphids and *Anoplolepis gracilipes*, *Crematogaster* sp., *Myrmicaria* sp., *Oecophylla smaragdina*, *Technamyrmex albipes* and *Tapinoma* sp. attended one aphid species each. Similarly, Joshi (2005) recorded 66 species of aphids of which 23 species of aphids were attended by eleven species

of ants. *Camponotus compressus* (F.) was found most abundant, associated with 15 aphid species. A total of 22 species of aphid natural enemies which is constituted by 14 species of predators and eight species of parasitoids were found associated with 16 and seven aphid species, respectively in the eastern dry zone of Karnataka.

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# A new species of *Cigaritis* Donzel, 1847 (Lycaenidae, Aphnaeinae) from the southern Western Ghats of Peninsular India

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**ABSTRACT:** A new species *Cigaritis meghamalaiensis* **sp. nov.** (Lycaenidae, Aphnaeinae) is described from the Meghamalai Hills of the Periyar landscape of the southern Western Ghats. Images of adults and illustrations of male genitalia are presented. Information on myrmecophilous immature stages is provided and its ecology is discussed. The new species is very distinct from all the known *Cigaritis* species in WG, and is diagnosed based on the following combination of characters–upper side of both wings marked extensively blue in males; discal and post-discal bands on forewing underside conjoined and lying parallel from their origin at the costa; post-basal band in hindwing underside continuous and not broken into three smaller bands and this post-basal band ends at vein1b, is not continued along it to reach discal band. The discal and post-discal bands on the underside of the forewing is conjoined and lying parallel from their origin at the costa which is a unique feature that distinguishes the new species from all other *Cigaritis* species occurring in Peninsular India and Sri Lanka. A key to all known species of *Cigaritis* from the Western Ghats is provided. © 2023 Association for Advancement of Entomology

**KEYWORDS:** Meghamalai Tiger Reserve, myrmecophily, new taxon, silverline, butterfly, crematogastor

## INTRODUCTION

The Silverlines are strong-winged lycaenids in the subfamily Aphnaeinae Distant, 1884. Evans (1932) treated Indian taxa of Aphnaeinae Distant, 1884 under *Aphnaeus* Hübner, [1819] in synonymy with *Spindasis* Wallengren, 1857 and considered

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*Cigaritis* Donzel 1847 as a senior synonym of *Apharitis* Riley 1925. Evans (1932) treated, species with prominent hindwing lobe and the tails at veins 1b and 2 nearly equal under *Spindasis* Wallengren, 1857 (=*Aphnaeus* Hübner, [1819]); and those species with ill-developed hindwing lobe and tail at v2 half as long as in vein 1 were treated under *Apharitis* Riley 1925 (=*Cigaritis* Donzel, 1847). Thus, the Western Ghats taxa were kept under the genera *Spindasis* and *Apharitis* by him. However, Heath (1997) synonymised *Apharitis* with *Spindasis*. The genus *Spindasis* was later synonymized with the genus *Cigaritis* Donzel, 1847, and *Aphnaeus* Hübner, [1819] was restricted to African taxa by Heath *et al*. (2002). Heath (1997), Heath *et al.* (2002), Heath and Pringle (2011), and later Boyle *et al.* (2015), using molecular data synonymized *Spindasis* with *Cigaritis*–the senior synonym. There are seven species of *Cigaritis* in the Western Ghats namely *C. vulcanus* (Fabricius, 1775)*, C. schistacea* (Moore, [1881])*, C. ictis ictis* (Hewitson, 1865)*, C. elima elima* (Moore, 1877)*, C. lohita lazularia* (Moore, 1881), *C. lilacinus* (Moore, 1884), and *C. abnormis* (Moore, [1884]). Of these, except *C. lilacinus* all others have been reported from the southern Western Ghats (Unpublished data – Sadasivan *et al.*). A distinct *Cigaritis* species in the high elevations of Periyar Tiger Reserve, Idukki district, Kerala in 2018, and its myrmecophilous immature stages was observed. On further exploration in 2021, this species was found to be common in Meghamalai (Megamalai) of Tamil Nadu and adjoining Periyar Tiger Reserve of Kerala (Fig.1). This *Cigaritis* species was found to be new to science and is described here.

## MATERIALS AND METHODS

The taxonomy of *Cigaritis* follows Evans (1932), Heath (1997), Heath *et al.* (2002), Heath and Pringle (2011) and Boyle *et al.* (2015). Identification of species follows Evans (1932), Wynter-Blyth (1957), and van der Poorten and van der Poorten (2018). Photographs of the specimens were taken with a Canon EOS 70D DSLR fitted with a 180mm macro lens and MPE 65 f 2.8 1–5x lens. The genitalia were studied by soaking overnight in KOH, then dissected under a stereo-zoom microscope (HEADZ Model HD81) and preserved in glycerol. Illustrations were drawn by the senior author using the stereo-zoom microscope. The length of the forewing (FW) is measured as the longest straight-line distance from the wing base to the wing tip following Van Hook *et al.* (2012). Terminology for the wing pattern follows Evans (1932) and genitalia descriptions follow Corbet & Pendlebury (1992). The holotype and paratypes will be deposited in the insect collection of the Zoological Survey of India (ZSI), Western Ghat Regional Centre (WGRC), Kozhikode, Kerala, and Bombay Natural History Society (BNHS), Mumbai.

### Abbreviations

| | |
|---|---|
| BNHS | Bombay Natural History Society |
| PTR | Periyar Tiger Reserve, Kerala |
| SMTR | Srivilliputhur-Meghamalai Tiger Reserve |
| TNHS | Travancore Nature History Society |
| UpF | Upperside of forewing |
| UnF | Underside of forewing |
| UpH | Upperside of hindwing |
| UnH | Underside of hindwing |
| WG | Western Ghats |
| WLS | Wildlife Sanctuary |
| ZSI | Zoological Survey of India, Kozhikode |

## RESULTS AND DISCUSSION

### Systematics

Family Lycaenidae Leach, 1815

Subfamily Aphnaeinae Distant, 1884

Genus *Cigaritis* Donzel, 1847

*Cigaritis meghamalaiensis* Sadasivan & Naicker **sp. nov.** LSID urn:lsid:zoobank.org:act:A8D4F48B-46E6-4692-81D4-0D8F2EE4DA03

*Holotype* (Figs. 2A–B): TLRG 1001; Kardana Estate, Meghamalai, Theni District, Tamil Nadu

State, India; Col. SRK; 15.vi.2021, 1400m ASL, from a private estate; dry pinned specimen; will be deposited in the insect collections of ZSI, WGRC, Kozhikode Kerala, India.

*Paratypes* (1 male and 2 females) (Figs. 2C–G): ♂: TLRG 1002; bearing the same data as the holotype; dry pinned specimen; will be deposited in the insect collections of BNHS, Mumbai, Maharashtra, India. ♀ TLRG 1003 and ♀ TLRG 1004 bearing the same data as the holotype; dry pinned specimens, will be deposited in the insect collections of ZSI, WGRC, Kozhikode, Kerala, India and BNHS, Mumbai, Maharashtra, India, respectively.

*Other materials* (observed, not collected): 2 ♀♀, 26. xii. 2016, Eravangalar, PTR, Kerala, 1400m ASL (KS & JJ); 21 ♂♂, and 14 ♀♀, 16.iv.2021, Kardana Estate, Chinnamanur Range, Theni District, Tamil Nadu, 1420m ASL (SRK); 6 ♂♂ and 4 ♀♀, 21.iv.2023, KSR Estate, Meghamalai Range, Theni District, Tamil Nadu, 1320m ASL (SRK).

Description of the Holotype (♂ TLRG 1001)

*Head.* Antennae dark brownish-black, inferolateral striations grey, and tip distinctly marked in pale orange-white; palpi dorsally blackish and covered with small thick whitish hairs ventrally; eyes grey with black speckling in life.

*Thorax.* dark grey bearing long pale greyish white hairs; legs very pale pinkish white, distally speckled in brown.

*Forewing*. Measures 16mm, costal margin and termen straight, apex minimally obtuse. Upperside with ground color velvety black, marked extensively with blue. Most of the spaces 1b and 2, three-fourths of space 3 from base, and just over half of space 4 are marked in metallic blue; a little over basal third of space 4 blue, rest of it black with a small blue spot at its middle; origin of space 5 blue; inferior half of cell blue. Entire costa marked broadly in black, this black border thickest at apex, then tapers on termen towards tornus; dorsum wholly marked in blue. Underside with ground color pale pinkish–brown, marked with a band at base of wing, and other long bands which are darker than ground color as follows–post-basal, discal, post-discal, sub-apical, submarginal bands pinkish–orange, centrally marked with silver scales, bordered with black; all long bands start at costa; post-basal band ends at origin of v2; discal band short, ends at origin of v3;


Fig. 1 Distribution of *Cigaritis meghamalaiensis* **sp. nov.** in Meghamalais of Periyar Landscape

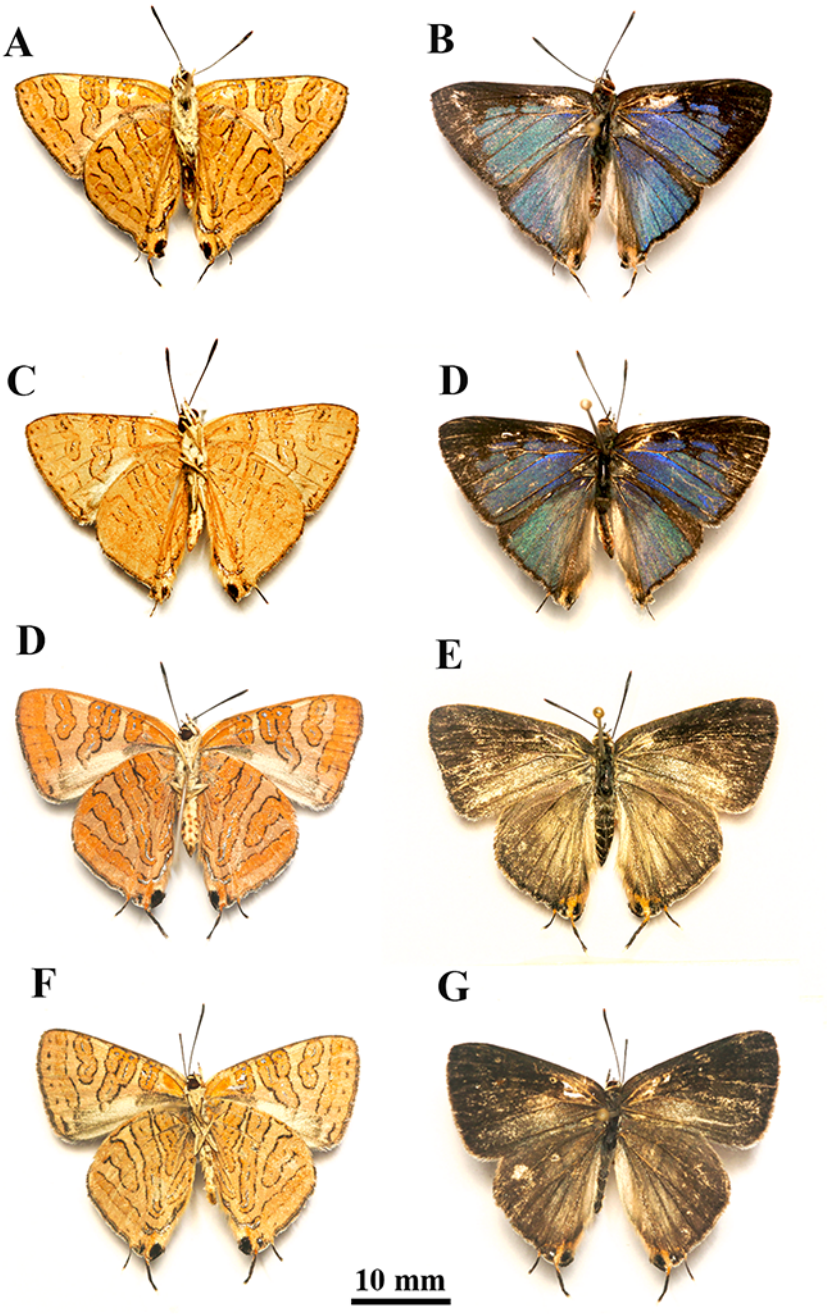


**Fig. 2** *Cigaritis meghamalaiensis* **sp. nov.** Images of the types and paratypes. A and B – TLRG 1001 Holotype male, A – dorsal and B ventral views; C and D –TLRG 1002, Paratype male, C – dorsal and D – ventral view; D and E – paratype female TLRG 1003, D – dorsal view and E – ventral view; F and G – paratype female TLRG 1004, F – dorsal view and G –ventral view. All images © Kalesh Sadasivan

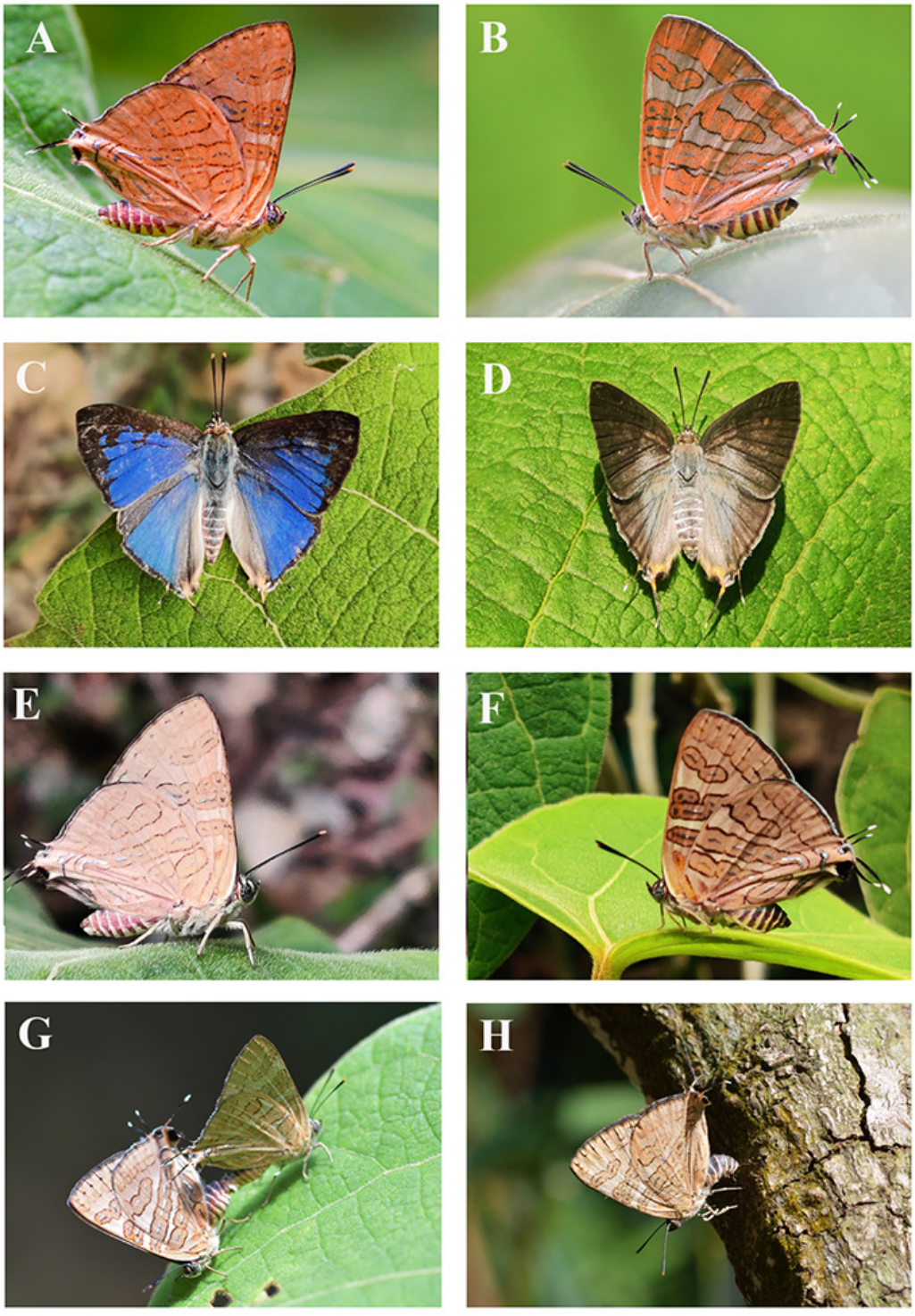


Fig. 3 *Cigaritis meghamalaiensis* **sp. nov.** Field images of males, females and seasonal forms. A–male. Typical color; B–female, typical color; C–male upperside; D–female upperside; E–dry season male underside; F–dry season female underside; G–mating; H–oviposition. All images © Ramasamy Naicker


**Fig. 4** *Cigaritis meghamalaiensis* **sp. nov.** Early stages, larval pens, and attending ants. A–freshly laid egg © Kalesh Sadasivan; B–larvae being attended by *Crematogaster* ants inside the larval pen © Kalesh Sadasivan; C–intermediate instar larvae and its attending ant © Kalesh Sadasivan; D–larval pen under the bark of a shola tree opened to reveal the walls (white arrows), and two larvae inside it marked 1 and 2 (blue arrows) © Jebin Jose

post-discal band consists of two conjoined bands, meeting at base of space 4; lower band turns basally and crosses into middle of space 1b; subapical band curved inwards ending at middle of space 3; submarginal band with its outer black border ill-defined, ends at v1b; a submarginal series of blackish-brown streaks on each space along termen; cilia brownish-black.

*Hindwing*. Upperside with ground color grey, whole of spaces 1c, 2, 3, 4, and cell marked in blue; origin of space 5 bears a small blue patch along vein 4; space Ib pale grey; costa broadly dark grey, termen narrowly marked in dark greyish black, dorsum pale greyish white with long pale grey hairs; whole of cell bears long bluish hairs; tornus pale orange white; tornal spot black; tails black, basal quarter pale orange-white, extreme tip white. Underside ground color as in forewing but slightly more orangish; short basal, longer–post-basal, discal, sub-apical and submarginal bands; longer bands originate at costa, run towards tornus; post-basal band continuous, not broken in three spots; discal band just crosses v1b, but does not reach tornus; post-basal and post-discal bands do not meet each other; sub-apical band crosses v3 at its middle; submarginal band just reaches 1b; all bands except the submarginal band bears central silver scales; silver scaling is sparse generally but well defined as a curvilinear streak running from end of submarginal band to middle of dorsal margin; tornal spots black, medial most spot almost twice as large as lateral.

*Abdomen.* Dorsally dark greyish-violet to violet-black, anterolaterally all segments bearing a cinnabar-red transverse streak that tapers towards ventrum, rest of segment yellowish-white; ventrally clothed in pale dirt white hairs.

*Male genitalia* (Fig. 6). Tegumen broad; uncus in lateral view broad, truncated, and flattened distally, appearing as a blunt tip tooth; dorsally tegumen and uncus appearing horseshoe shaped with a u-shaped gap separating the halves; uncus tip flattened and appearing spatulated in dorsal view; subuncal process shorter than uncus, thin, and directed towards opposite side and tips pointing posterolaterally; vinculum moderately thick, with a shallow concavity cephalad; saccus thicker than vinculum; caudal plate of saccus absent; valva with a middorsal auricular process; dorsal process of valva long triangular, curved inferomedially and its tip directed posteroinferiorly, with respect to rest of valva; aedeagus as in fig. 6D, with its tip bearing a sectorized triangular plate, edges of which is toothed.

**Description of female** (Figs. 2D–G; 3B, D, F, H; 5D): A rounded greyish form of male without azure-blue upper sides. Wing span 35–38mm.

*Head* and *Thorax.* As in males.

*Forewing*. Measures 17mm, colour dark brownish-black with pale greyish-blue scaling in basal two-thirds of space 1 and base of space 2; termen rounded, apex rounded in comparison to males; cilia greyish-brown. All bands and marking as in male but sub-apical band more angulated towards termen in females in comparison to males.

*Hindwing*. A paler shade of forewing, greyish-brown, discal area clothed heavily in long bluish-grey hairs; tornal region yellowish-orange bearing a large medial black tornal spot and a smaller one laterally between tails; tails colored as in males. Cilia grey.

*Abdomen.* As in males with reddish lateral markings less prominent, speckled in black scales.

***Variation:*** Not much variation was observed in the adults, except for the extent of silver scaling inside the bands and the lesser extent of reddish-orange hue on the underside in DSF individuals. Male genitalia is consistent. Forewing length was slightly variable to some extent, in males 15–17mm and females 16–18mm. On dry preservation, the blue shade on wings developed a greenish tinge.

***Etymology:*** The new species is named after the Meghamalai region where it was discovered. Meghamalai means ‘cloud mountain’, reflecting the montane habitat of this very local species, which is restricted to the sub-tropical evergreen ‘sholas’ or cloud forests of the Periyar landscape. We suggest the common name ‘Cloud-forest Silverline’.

***Ecology:*** Flight period observed was from

December to June. The butterfly, unlike its congeners, is very uninclined to fly and often falls easy prey to predators like *Monilesaurus acanthocephalus* Pal, Vijayakumar, Shanker, Jayarajan & Deepak, 2018 (Squamata, Agamidae). The butterfly is restricted to the sub-tropical evergreen forests and keeps to the forest edges where they perform mating and basking. The females were observed flying around trees occupied by *Crematogaster* ants. Another species that flies in the same elevation is *Cigaritis lohita.* The adults of *C. lohita* are not uncommon on the southern WG and their larvae have been observed on various plant families like Cannabaceae, Euphorbiaceae, Loranthaceae, Mimosaceae, and Myrsinaceae. However, the locally preferred species is *Maesa indica* (Roxb.) A. DC. (Myrsinaceae), and the larvae were seen attended by *Crematogaster rothneyi civa* Forel, 1902.

***Immature stages and myrmecophily:*** Mating was noted in April (Fig. 3G), and oviposition was noted in mid-April and late December. Females lay eggs (Fig. 4A) on dry bark of trees such as *Neolitsea* (Benth. & Hook. f.) Merr. (Lauraceae) inside the shola and shrubs like *Clerodendrum infortunatum* L. on shola edges (Figs. 3H; 5D), invariably in the presence of the ant *Crematogaster wroughtonii* Forel, 1902 and their nests (Figs. 4 B–D). Oviposition was observed between 11.00 a.m. and 1.30 p.m. Eggs are laid on stems inside ant nests, dark crevices on tree trunks and fallen branches with moss and lichen. The females preferred trees and shrubs whose stem diameter was less than 20 cm, and eggs were laid at heights of 1 meter or less from the ground well away from any foliage. Immature stages were observed inside *Crematogaster* ant larval pens (Fig. 4D), under the bark of *Neolitsea cassia* (L.), Kosterm., a shola tree at Eravangalar in Periyar (1400m ASL). Each larval pen contained 3–4 larvae in various stages of development (Fig. 4D). We observed that the larvae scrape and eat the soft bark of the tree and are sheltered under the hard bark in small pens created by *Crematogaster* ants, possibly with the droppings of the caterpillars and vegetable matter (Fig. 4D). These larval pens were in the main trunk of the tree at a height of 1.5–2m from the ground, very far away from any leaves of the tree. The ant nests were very far away from these larval pens, and thus the possibility of feeding by trophallaxis is suggested rather than the larvae being parasitic or predatory on these ant and their broods.

Of the seven species of *Cigaritis* known from the Western Ghats, six are reported on the southern Western Ghats namely *C. vulcanus*, *C. schistacea*, *C. ictis ictis, C. elima elima, C. lohita lazularia*, and *C. abnormis*. Of these, as per our field observation, *C. vulcanus* is a ubiquitous species seen in all elevations from sea coasts to 1200m, *C. schistacea* is an uncommon midland species (200–1200m), while *C. ictis* and *C. elima* are distributed below 800m, especially on the drier eastern slopes and *C. lohita* is a species which is found from the seacoast to about 1800m on the WG. *Crematogaster abnormis* is reported to occur on the lower eastern slopes of Coorg, Wayanad, Nilgiri, and Anamalai landscapes below 800m. Only *C. lohita* was shares the elevational habitat of the new species. The *'unidentified' Cigaritis* sp. mentioned in Sujitha *et al.* (2023) is described here as new to science and the myrmecophilous association with *Crematogaster wroughtonii* is confirmed. The new species is very distinct from all the known *Cigaritis* species in WG, and is diagnosed based on the following combination of characters–upper side of both wings marked extensively in blue; discal and post-discal bands on forewing underside conjoined and lying parallel from their origin at the costa; post-basal band in hindwing underside continuous and not broken into three smaller bands and this post-basal band ends at vein1b, is not continued along it to reach discal band. The presence of extensive blue coloration on Fw readily separates this new species from *C. elima elima*, *C. ictis ictis*, *C. schistacea*, and *C. vulcanus*, all of which have some form of orange stripes on the forewing. In addition, the unbroken post-basal band UnH distinguishes the new species from *C. elima elima*, *C. ictis ictis* as well as *C. abnormis,* and *C. lilacinus*. The hindwing underside post-basal band ends at vein1b, not continuing along it to reach the discal band separates the new taxon from *C. lohita.*

The discal and post-discal bands UnF conjoined and


Fig. 5 *Cigaritis meghamalaiensis* **sp. nov.,** habitat and host plants. A–Sub-tropical Evergreen forests of Meghamalais (1400m ASL) © Ramasamy Naicker; B–*Neolitsea cassia* (L.), Kosterm., a host tree © Kalesh Sadasivan; C–typical climate inside the misty cloud forests © Jebin Jose; D–female ovipositing inside *Crematogaster* nest on *Clerodendrum infortunatum* L. © Ramasamy Naicker

lying parallel from their origin at the costa is a unique feature that distinguishes *C. meghamalaiensis* **sp. nov.** from all known species of *Cigaritis* in Peninsular India and Sri Lanka. The discovery of a new species of *Cigaritis* from the southern Western Ghats reiterates the possibility of discovering new species which may have sought refuge in the montane sholas and cloud forests, which are under severe anthropogenic stress. The nature of myrmecophilous interaction needs to be studied in detail, and the possibility of finding this new species must be kept in consideration wherever the attending host ant *C. wroughtonii* occurs in the Western Ghats.

**Key to *Cigaritis* Donzel, 1847 of Western Ghats Modified from Wynter-Blyth (1957), based on the males**

1. UnH the second band from the post-basal band continued along v1b to meet the discal band

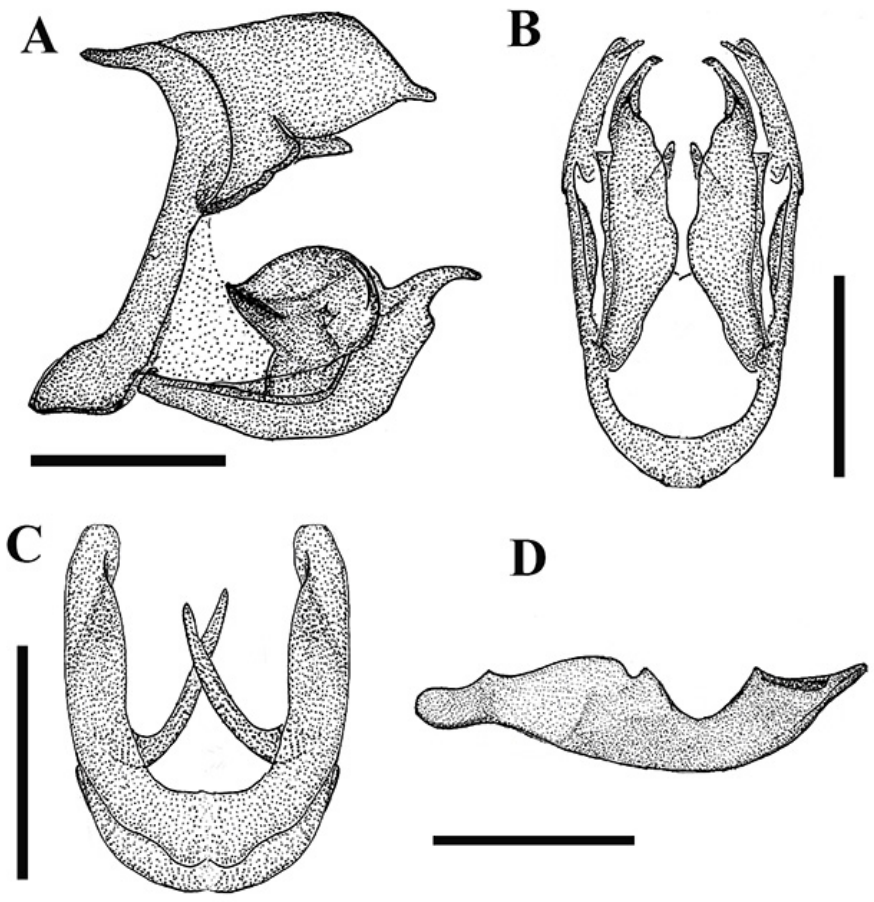


Fig. 6 *Cigaritis meghamalaiensis* **sp. nov.,** Illustration of male genitalia (scale bar 1.5 mm). A–right lateral view of the genitalia with the aedeagus removed; B–ventral view of the valva; C–dorsal view of the uncus; D–right lateral view of the aedeagus

near the tornus; below creamy yellow to cinnamon red, bands black to red; male above azure blue (Figs. 7A, B) .....*Cigaritis lohita lazularia*

– UnH post-basal band ends at vein 1b, not continue along it to reach the discal band….....….....2

2. UnH the post-basal band continuous as judged by continuity of outer black margin of that band….....3

– UnH post-basal band from base broken into 3 spots and not continuous….....5

3. UpF and UpH with orange stripes, in grey background; discal and post discal bands always separate at their origin at the costa.....4

– UpF and UpH are marked extensively in blue on a black background; UnF discal and post discal bands conjoined and lying parallel from their origin at the costa. (Figs. 7G, H) .....*Cigaritis meghamalaiensis* **sp. nov.**

4. A conspicuous small patch of blue scales near the orange tornal patch on UpH; orange patches on UpF apex restricted. (Figs. 8C, D).....*Cigaritis schistacea*

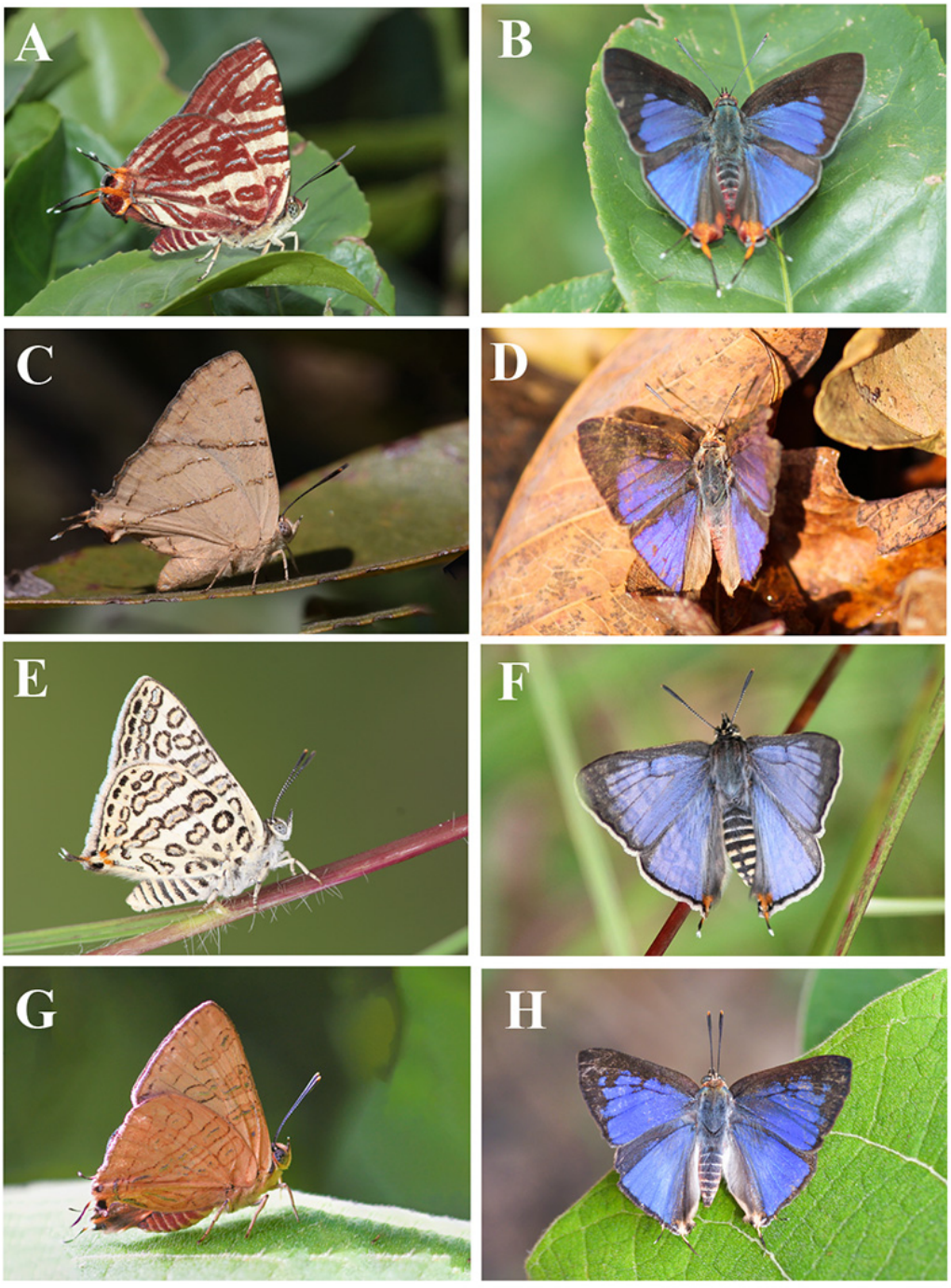


Fig. 7 *Cigaritis* of Western Ghats. A–*Cigaritis lohita* male ventral view © Kalesh Sadasivan; B–*Cigaritis lohita* male dorsal view © Kalesh Sadasivan; C–*C. abnormis* male ventral view © Milind Bhakare; D–*C. abnormis* female dorsal view © Prateik More; E–*C. lilacinus* male ventral view © Kalesh Sadasivan; F–*C. lilacinus* male dorsal view © Haneesh KM: G–*C. meghamalaiensis* **sp. nov.** male ventral view © Ramasamy Naicker; H–*C. meghamalaiensis* **sp. nov.** male dorsal view © Ramasamy Naicker

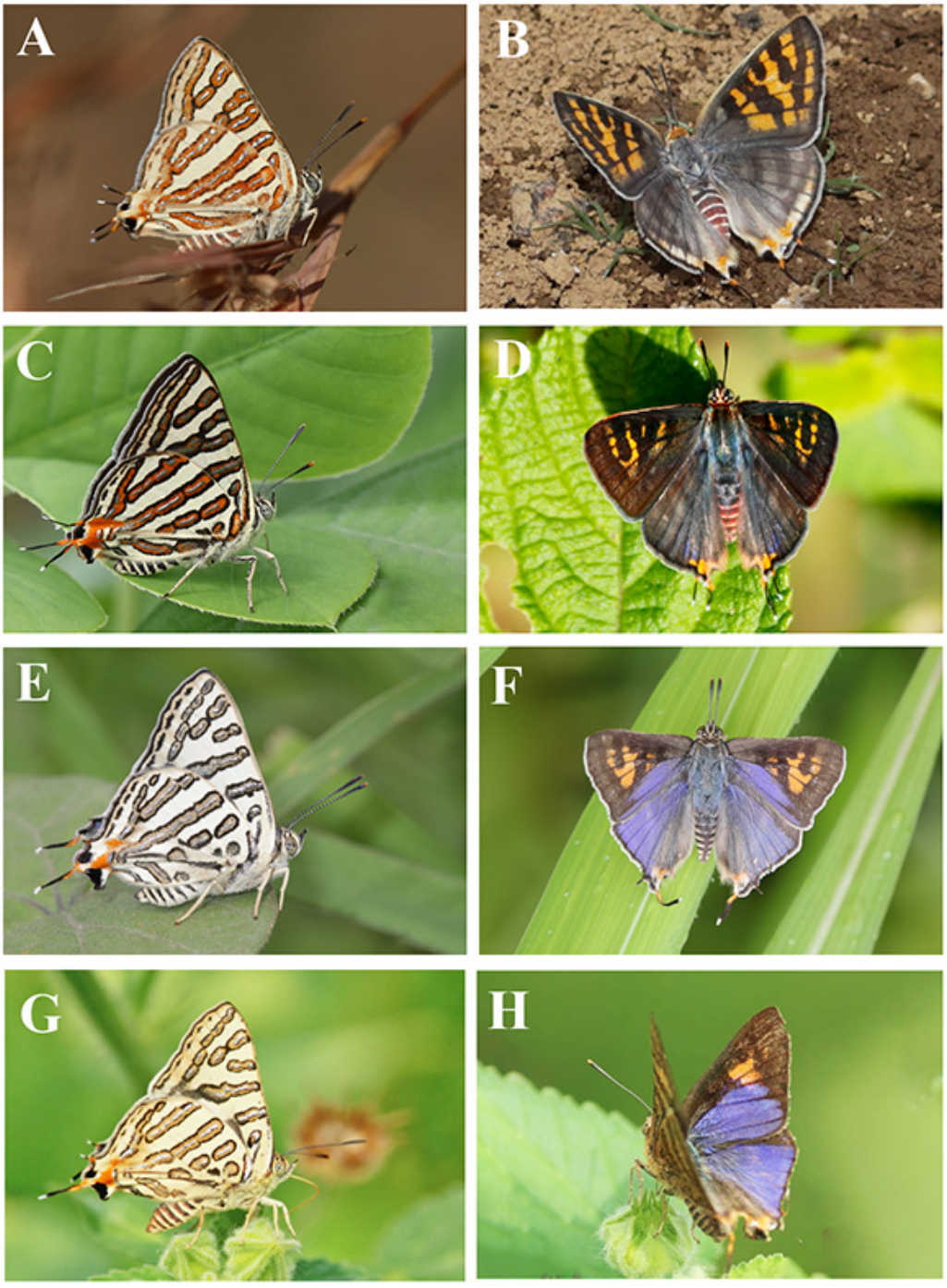


Fig. 8 *Cigaritis* of Western Ghats. A–*Cigaritis vulcanus* male ventral view © Milind Bhakare, B–*C. vulcanus* male dorsal view © Milind Bhakare; C–*C. schistacea* male ventral view © Milind Bhakare; D–*C. schistacea* male dorsal view © Jebin Jose; E–*C. ictis* male ventral view © Milind Bhakare; F–*C. ictis* male dorsal view © Milind Bhakare; G–*C. elima* male ventral view © Haneesh KM; H–*C. elima* male dorsal view © Haneesh KM

– UpH without blue patch; orange patches on the UpF extensive. (Figs. 8A, B) .....*Cigaritis vulcanus*

5. UnH the lower two basal bands conspicuous, UpF apical orange patch present in males, above bright blue; three seasonal forms, pale yellow WSF with dark edged dark yellow bands, cinnamon or khaki autumn form with black-edged bands or khaki spring form with bands defined by silver lines and black margins absent….....6

– UnH the lower two basal bands inconspicuous and markings abnormal and narrow and only central bands are seen; male above only lightly blue, no orange apical patches; dull reddish brown below; forewing apex violet-brown and generally grey otherwise (Figs. 7C, D) .....*Cigaritis abnormis*

6. UpF with orange patches in brown background……...…….7

– UpF lilac blue in grey background (Figs. 7E, F)……..…….....……*Cigaritis lilacinus*

7. UpF apical orange patch is developed and conspicuous; the basal blue patch does not reach vein 2; last band (postdiscal) on UnH meets or leans towards the submarginal band. (Figs. 8E, F) .....*Cigaritis ictis ictis*

– UpF apical orange patch restricted, basal blue patch reaches vein 2; the last band (postdiscal) on UnH never meets and leans away from the submarginal band. (Figs. 8G, H) .....*Cigaritis elima elima*

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# Redescription of *Cyphochilus niveosquamosus* (Blanchard, 1851) (Coleoptera, Scarabaeidae, Melolonthinae) with notes on two species of the genus from India

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**ABSTRACT:** *Cyphochilus niveosquamosus* (Blanchard, 1851) from India is redescribed. The habitus and parameres of *C. candidus* (Olivier, 1789) and *C. septentrionalis* Waterhouse, 1867 redescribed by Sabatinelli (2020) are also illustrated. The species are also redescribed based on the newly examined specimens in this study. A checklist with distribution of eight species of *Cyphochilus* from India is provided.


**KEY WORDS**: *Cyphochilus candidus, C. septentrionalis,* habitus, parameres, checklist

## INTRODUCTION

The genus *Cyphochilus* Waterhouse, 1867 is characterized by the strongly asymmetric labrum among Scarabaeidae. The beetles possess an exceptional ability to scatter visible light from their scales to produce brilliant whiteness (Vukusic *et al*., 2007). Sabatinelli, (2020) revised the "Section II" of the genus defined by Waterhouse (1867) as "the species having the mesosternum armed by a spur" and redescribed *C. candidus* (Olivier, 1789) and *C. septentrionalis* Waterhouse, 1867 for the first time and recognised two new species *viz., C. gandhii* Sabatinelli, 2020 and *C. satyarthii* Sabatinelli, 2020 from north India. So far, 64 species of the genus *Cyphochilus* have been described worldwide (Sabatinelli, 2020a, b, c; Sabatinelli and Pham, 2021; Zhao, 2021) and no information on the south Indian species except for the original description of species is available. Despite, being a melolonthine beetle, hitherto none of the members of this genus have been reported as pest of any crop. In this paper, *C. niveosquamosus* (Blanchard, 1851) is redescribed from south India along with new locality record and notes of the two north Indian species, *C. candidus* (Olivier, 1789) and *C. septentrionalis* Waterhouse, 1867.

## MATERIALS AND METHODS

The specimens were examined under a Nikon SMZ-1000 stereo binocular microscope for the study of the external morphology. For the study of male genitalia, the abdomen was separated from the rest of the body, cleaned in hot water and later macerated in hot KOH (10%), washed in distilled water and placed in glycerine for separating genitalia and further observation and imaging. The dissected

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genitalia and the rest of the specimen were suitably marked to ensure their association.

Images were made using Canon 7D camera with 100 mm and MPE65 mm lenses, series of images were taken at different depths and were subsequently stacked using Zerene Stacker software 1.04 to obtain high resolution images and grouped in plates using Adobe Photoshop CS2. All the specimens studied are deposited in the Department of Entomology, University of Agricultural Sciences, Bengaluru, abbreviated in the material examined section as UASB.

Abbreviations: Characters, measurements, mensural procedures, and ratios are those used by Sabatinelli (2020).

BL: Body length (from anterior margin of clypeus to apex of elytra, in dorsal view)

BW: Body width (across the elytral humeri, in dorsal view)

BWX: Greatest body width (across the elytral maximal width, in dorsal view)

CW/L: Clypeal ratio (width measured along clypeo-frontal suture divided by greatest length of clypeus)

F/O: Interocular ratio (minimum frons width across eyes divided by transverse compound eye diameter in dorsal view)

A2-7L/CL: Antennal ratio (derived from length of basal segments 2-7 divided by antennal club length)

PnW/L: Pronotal ratio (pronotal greatest width divided pronotal length along midline in dorsal view)

MstL: Mesosternal process length, in lateral view

## RESULTS AND DISCUSSION

### Genus *Cyphochilus* Waterhouse, 1867

**Type species:** *Melolontha candida* Olivier, 1789 by subsequent designation by Medvedev, 1951: 231.

Diagnosis: Principal diagnostic characters of *Cyphochilus* are: Labrum strongly asymmetric and body surface covered with scales.

*Cyphochilus niveosquamosus* (Blanchard, 1851) Figs. 1A-D, 2 A-D

*Leucopholis niveosquamosa* Blanchard, 1851: 158.

Waterhouse, 1867: 142 (*Cyphochilus*)

Material examined: INDIA: Tamil Nadu: 4♂, 1♀, Valparai: Near UPASI, Tea Research Station, 1062m, 10°16'N; 76°58'E, 02.vii.2014, leg. H.M. Yeshwanth (UASB).

Description: *Size* – BL: 17.74mm, BW: 7.81mm, BWX: 8.8mm at mid-point of elytra. *Colour* – Integument dark reddish brown including antennal club and legs; dorsal surface with uniformly pale white lanceolate scales each originating from a small puncture, scales denser along lateral margins of pronotum and elytra. *Head* – CW/L: 2.72; clypeus subrectangular, anterior margin straight, slightly reflexed; clypeo-frontal suture distinct; eyes prominent (F/O: 3); frons with an indentation behind the clypeo-frontal suture; antennal club longer than antennomeres 2-7 combined (A2-7/CL: 0.54). *Pronotum*–Transverse (PnW/L: 2), strongly convex, anterior margin concave, posterior margin arched at middle, anterior angles slightly obtuse, posterior angles angularly rounded, lateral margin angularly protruded almost at midlength, anterior half of the lateral margin devoid of scales. *Scutellum*–Semi-circular, covered with scales of varied size usually smaller than scales on elytra. *Elytra*–Elongate, parallel sided, irregularly punctate, stria not recognizable, suture raised, each elytron with two distinct and one feeble costae running parallel to each other. *Pygidium*–Broad, apex arched, dorsal surface with uniform small scales, apex and sides with a series of short hairs. *Thoracic sterna*–With long yellowish brown pubescence and sparse spiniform scales; prosternal process tubercular; mesosternal process peg-like, narrowed and rounded anteriorly, ventral surface slightly convex with a median longitudinal carina extending from a short distance before apex to midlength and dorsal apical surface flat (MstL: 1.18mm), ventrally glabrous. *Abdomen*–Ventrites with sparse scales,

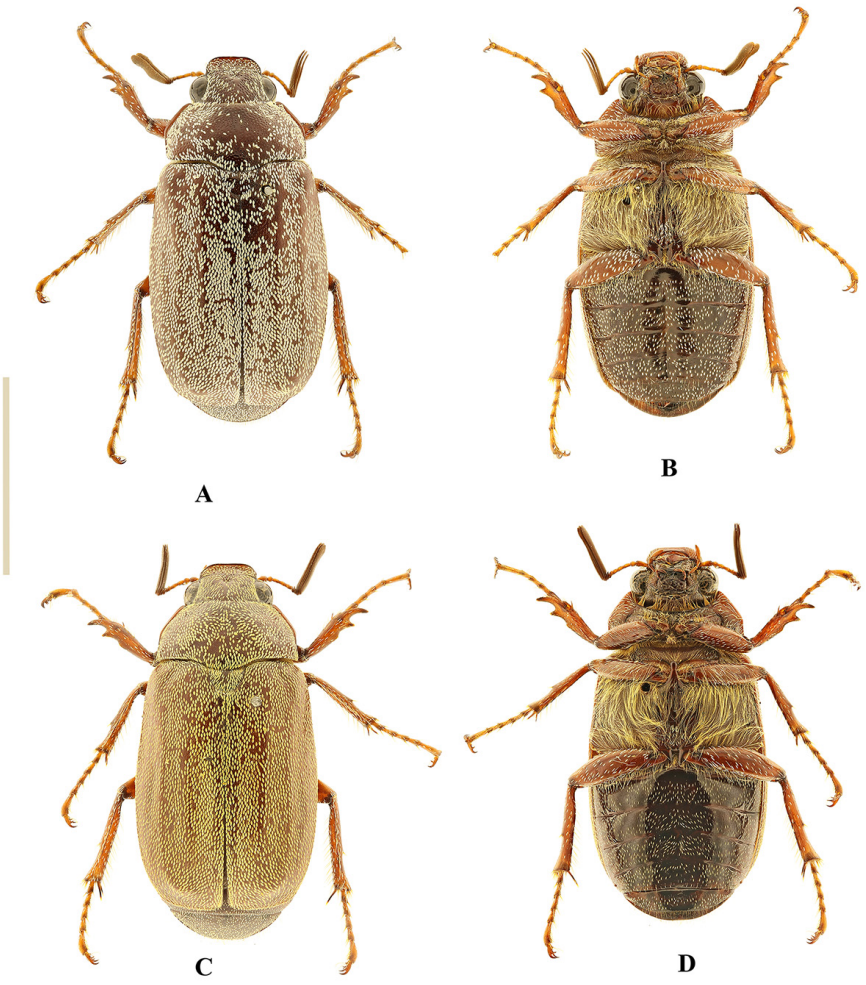


Fig.1 *Cyphochilus niveosquamosus* (Blanchard, 1851), A-B: Male with white scales; A: Dorsal view, B: Ventral view, C-D: Male with yellow scales; C: Dorsal view, D: Ventral view

intersegmental suture prominent, border of 5th sternite with a row of short hairs, 1-4 ventrites glabrous. *Legs*–Protibia tridentate, apical tooth acute; subapical tooth triangular and positioned at same level as inner apex of protibia. *Aedeagus*–Basal piece 1.4× longer than paramere, medially longitudinally keeled, basi-dorsal angle smoothly curved, in lateral view gradually widened distally, lateral ventral margin in distal 1/3rd curved dorsally. Parameres fused till very close to apex, symmetrical, in lateral view gradually narrowed distally in basal 0.8 length then widened, distal apex bifid in lateral view, ventro-apical angles of lower branch projected and pointed, dorso-apical angle of upper branch rounded.

Variability: BL; 17.17–19.7mm (x=18.5mm, n=5); vestiture of scales pale yellow.

Remarks: Blanchard (1851) reported *Leucopholis niveosquamosa* from Mysore (erstwhile princely state). Here the species is recorded from Valparai (Tamil Nadu, south India). The specimens collected in this study are much shorter (around 18mm) than the specimens studied by Blanchard (1851) and Waterhouse (1866-67) which ranged from 24–25mm.

***Cyphochilus candidus* (Olivier, 1789)**
Figs. 3 A-E, 4 A-E

*Melolontha candida* Olivier, 1789: 15.

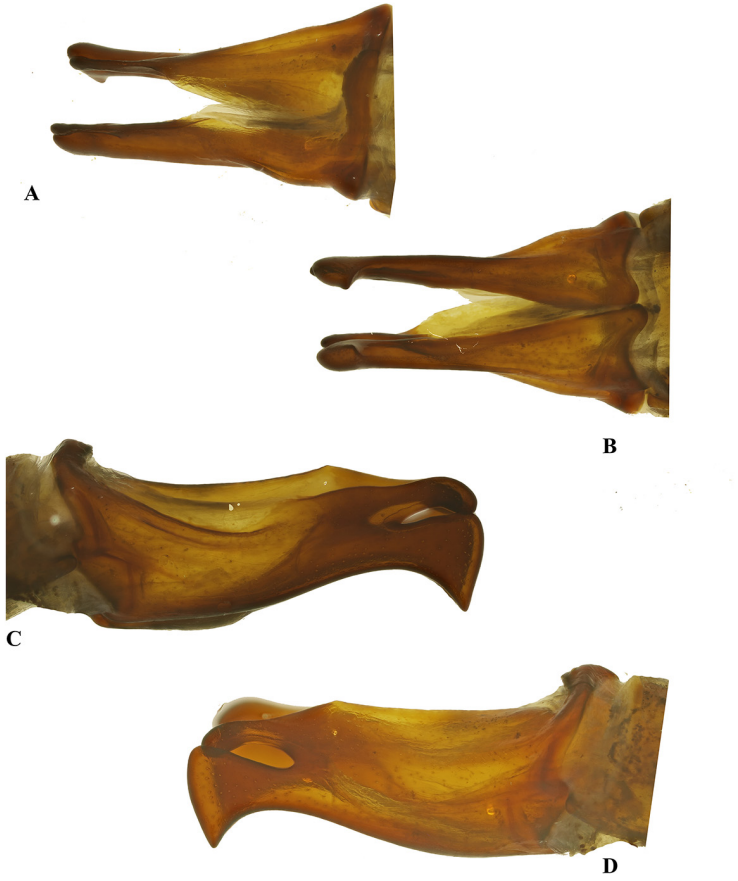


Fig. 2 *Cyphochilus niveosquamosus* (Blanchard, 1851)
Aedeagus - A: Dorsal view, B: Ventral view, C - D: Lateral views of paramere

Material Examined: INDIA: Meghalaya: 4♂, Ri-Bhoi, ICAR-Research Complex for North Eastern Hill Region; Umiam, 1031m, 25°41'N; 91°55'E, 3.vi.2013, leg. H. M. Yeshwanth;1♂, Shillong, 1996; leg. S.K. Gangwar;1♂, East Khasi hills: Mawkdok, 6.vi.2013, leg. H.M. Yeshwanth (UASB).

Description: *Size* – BL: 23.6mm, BW: 10.66mm, BWX: 12.08mm at mid-point of elytra. *Colour* – Integument dark reddish brown including antennal club and legs; dorsal surface with pale yellowish scale, each scale arising from a small puncture, denser along pronotal margins and sides of elytra. *Head* – CW/L: 2.67; anterior margin of clypeus straight, slightly reflexed; eyes prominent (F/O: 4.8); frons with a subtriangular depression behind clypeo-frontal suture; antennal club longer than antennomeres 2-7 combined (A2-7/CL: 0.65), one of the setae on antennomere 7 approximately equal to 1/3 as long as antennal club. *Pronotum*– Transverse (PnW/L: 2), strongly convex, anterior margin concave, posterior margin arched at middle, anterior angles right angled, posterior angles angularly rounded and obtuse. *Scutellum* – Semi-circular densely covered with scales. *Elytra*–No visible stria or costa on elytra. *Pygidium*–Convex, apex deeply notched and apical margin reflexed, dorsal surface covered with scales. *Thoracic sterna* – With long yellowish brown pubescence and sparse scales; prosternal process tubercular; mesosternal process well developed (MstL: 2.4mm), acute, ventrally glabrous. *Abdomen* – Sternites with sparse scales, medioapical part of 8$^{th}$ sternite glabrous. *Legs* –Protibia tridentate, apical tooth acute; subapical tooth triangular and positioned at same level as inner apex of protibia.

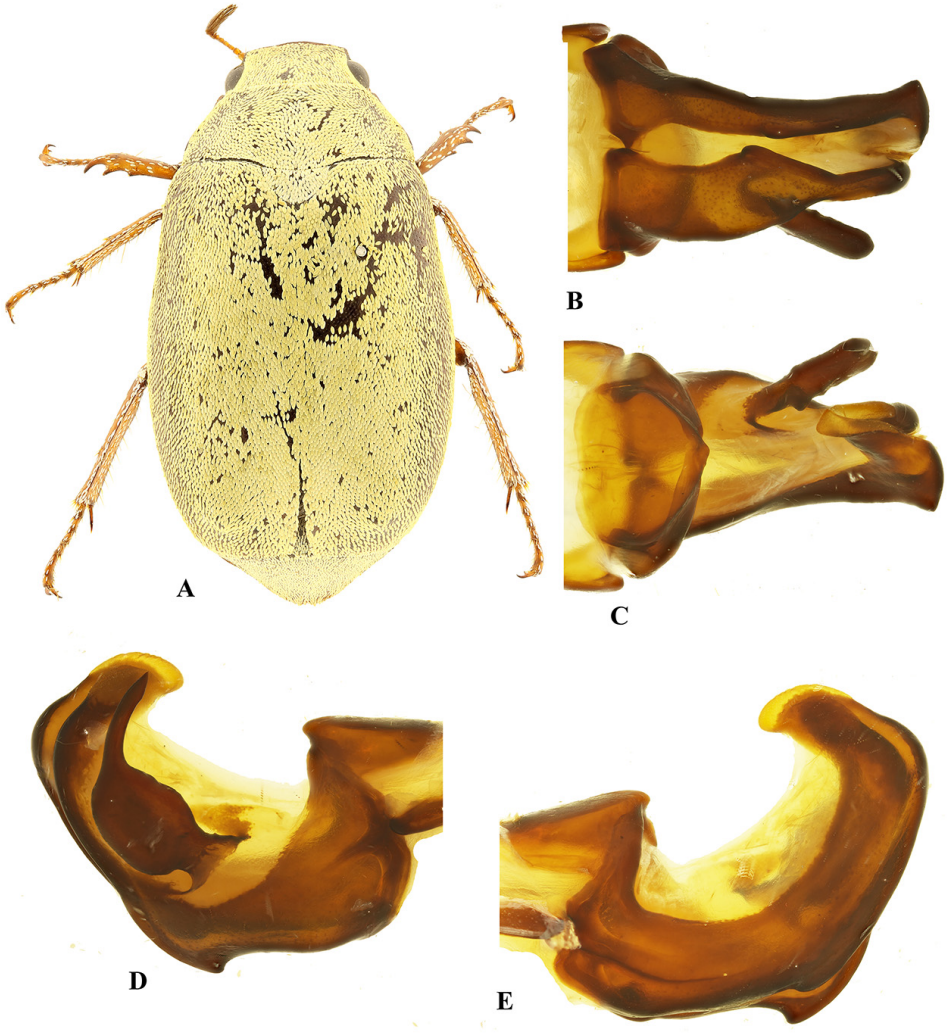


Fig. 3 *Cyphochilus candidus* (Olivier, 1789) , A: Dorsal view (Male with yellow scales); Aedeagus - B: Ventral view, C: Dorsal view, D: Left paramere with a spur, E: Right paramere

*Aedeagus* – Basal piece along with parameres 'S' shaped, about 1.3× as long as paramere, without median keel. Parameres asymmetrical, strongly curved dorsally, inner margin of parameres dorsally projected at midline, projection of left paramere more prominent than right one, left paramere with a spur, broad with rounded margin in basal half, strongly constricted and spine like in distal half.

Variability: BL: 22.32–24.71mm (x=23.5mm, n=3); vestiture of scales white (Fig. 4A-E). Shape of the spur on left paramere varied from subovate to subrectangular.

***Cyphochilus septentrionalis* Waterhouse, 1867,** Figs. 5 A-E

*Cyphochilus septentrionalis* Waterhouse, 1867: 141.

*Cyphochilus pygidialis* Nonfried, 1893: 332. Synonymised by Sabatinelli, 2020

*Cyphochilus pygidialis* v. *angeri* Nonfried, 1893: 333. Synonymised by Sabatinelli, 2020

Material studied: INDIA: Manipur: 1♂, Ukhrul; 1647 m, 25°06'N; 94°21'E, 23.viii.2014, leg. H.M. Yeshwanth (UASB).

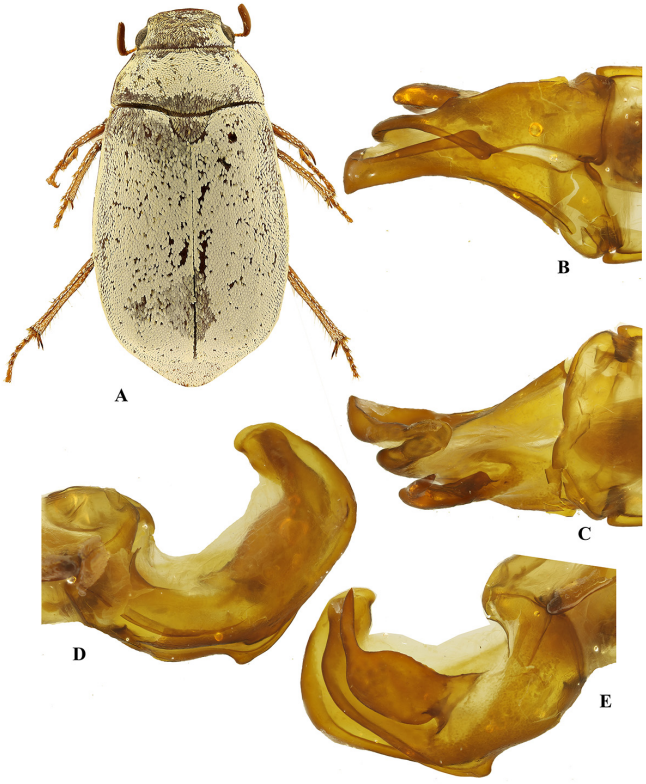


Fig. 4 *Cyphochilus candidus* (Olivier, 1789) , A: Dorsal view (Male with white scales); Aedeagus - B: Ventral view, C: Dorsal view, D: Right paramere, E: Left paramere with a spur

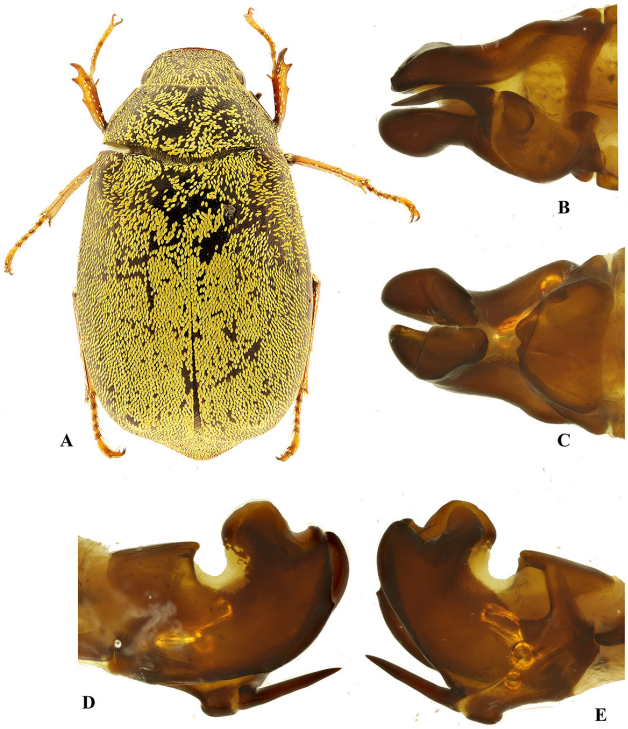


Fig. 5 *Cyphochilus septentrionalis* Waterhouse, 1867 , A: Dorsal view (Male); Aedeagus - B: Ventral view, C: Dorsal view, D: Right paramere, E: Left paramere with a spine

**Description**: *Size* – BL: 23.44mm, BW: 10mm, BWX: 11.87mm at mid-point of elytra. *Colour* – Integument dark reddish brown including antennal club and legs; dorsal surface with uniformly yellow lanceolate scales, each scale originating from a small puncture, scales on clypeus, frons, pronotal and elytral disc comparatively larger; sides of pronotum, lateral and apical margin of elytra with smaller scales. *Head* – CW/L: 2.85; anterior margin of clypeus straight, slightly reflexed; clypeo-frontal suture feeble; eyes not prominent (F/O: 7.9); a subtriangular depression on frons just behind clypeo-frontal suture distinct; antennal club slightly shorter than antennomeres 2-7 combined (A2-7/CL: 1.12). *Pronotum* – Transverse (PnW/L: 2), strongly convex, anterior margin concave, posterior margin arched at middle, anterior angles almost right angled, posterior angles conically rounded and obtuse angled. *Scutellum* – Semi-circular, sparsely covered with scales. *Elytra* – Without visible stria or costa. *Pygidium* – Convex, apex rounded and apical margin reflexed, dorsal surface with scales. *Thoracic sterna* – With long yellowish brown pubescence and spiniform scales; prosternal process tubercular; mesosternal process well developed (MstL: 2.6 mm), acute, ventrally glabrous. *Abdomen*–Sternites with sparse scales, medioapical part of 8$^{th}$ sternite glabrous. *Legs* – Protibia tridentate, apical tooth acute; subapical tooth triangular and positioned at same level as inner apex of protibia. *Aedeagus* – Basal piece 1.33× longer than parameres and without median keel. Parameres asymmetrical, fused at base and dorsally curved, a posteriorly directed pointed spine on ventral inner margin at midlength of left paramere and a short tooth-like projection on ventral inner margin of right paramere subapically, right paramere concavely excavated along the inner margin subapically, left paramere slightly sinuate along inner margin subapically.

Remarks: This species resembles *C. candidus* (Olivier, 1789) externally but can be differentiated by the aedeagus. *C. candidus* has a subovate to subrectangular spur with distal attenuated process on the outer surface of left paramere, whereas *C. septentrionalis* has a posteriorly directed pointed spur on the ventral inner margin.

Updated checklist of species of *Cyphochilus* Waterhouse, 1867 known from India

*C. candidus* (Olivier, 1789) –India: Sikkim, West Bengal, Assam, Meghalaya, Manipur

*C. gandhii* Sabatinelli, 2020– India: West Bengal, Sikkim, Assam, Arunachal Pradesh

*C. manipurensis* Nonfried, 1893 – India: Manipur

*C. niveosquamosus* (Blanchard, 1851) – India: Karnataka, Tamil Nadu

*C. oberthueri* Brenske, 1903 – India: Tamil Nadu

*C. satyarthii* Sabatinelli, 2020– India: Sikkim, West Bengal

*C. septentrionalis* Waterhouse, 1867 – India: Uttar Pradesh, Sikkim, Assam; Meghalaya, West Bengal and Nagaland

*C. waterhousei* Brenske, 1903 – south India

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# Evaluation of pongamia oil soap against red spider mites, *Tetranychus urticae* Koch and its effect on mite predators in brinjal ecosystem

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**ABSTRACT**: The efficacy of pongamia oil soap was tested on red spider mites and on its predators (rove beetle, *Oligota* sp. and predatory gall midge, *Feltiella acarisuga* Vallot). Pongamia oil soap at 0.6, 1, 2 and 3 per cent along with neem oil 0.6 per cent, soap solution 0.5 per cent and thiamethoxam 25 WG were investigated and observations were recorded on, a day prior to and, 1, 3, 5, 7 and 14 days after spray application. The results revealed that pongamia oil soap @ 3, 2 and 1 per cent shown mite population reduction up to 86.50, 84.57 and 79.27 per cent respectively. Richness of mite predators didn't vary significantly at one day after spray and the maximum population was observed in soap solution (4.33/5 plants). Population of mite predators was relatively high at 14 days after spray with soap solution (35.67 per five plants) which was at par with pongamia oil soap @ 0.6, 1 and 2 per cent.


**KEY WORDS:** Rove beetle, predatory gall midge, thiamethoxam, neem oil, soap solution

## INTRODUCTION

Brinjal is one of the major vegetables crops in India which positions second in brinjal (*Solanum melongena* L.) production with 12.80 MT of production (National Horticulture Board, 2018). There are 140 different types of insect and non-insect pests have a history of damaging the crop. The destructive pest of brinjal is fruit and shoot borer (BFSB), *Leucinodes orbonalis* Guenee (Lepidoptera, Crambidae) which causes enormous yield loss of as high as 70-92 per cent (Rosaiah, 2001). When infestation levels are high, sucking pests like red spider mites; *Tetranychus urticae* Koch (Tetranychidae, Acari) dramatically lower crop output and inflict damage.

*Millettia pinnata* (L.) Pierre, often known as "pongam," "Indian beech," or "karanj," is a multifunctional tree that is especially prized for the oil that is extracted from its seeds (27–40% oil). The primary furanoflavones responsible for karanj oil's insecticidal effects on pests, are karanjin, pongapin, kanjone, and diketone pongamol (Bringi, 1987). Different pongamia extracts shown antifeedant and oviposition-deterrent efficacy against various agricultural pests (Kumar *et al*., 2006). A field experiment was conducted to

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evaluate the efficacy of four different concentrations of soap formulation of pungam (Milletia/Pongamia) oil against the red spider mite, *Tetranychus urticae* Koch and its effects against its natural enemies, (rove beetle, *Oligota* sp. and a predatory gall midge, *Feltiella acarisuga* Vallot population), in an eggplant ecosystem.

## MATERIALS AND METHODS

Pungam oil soap made based on the technology used in "Ready to use Neem Oil Garlic Soap", the biocide approved by Kerala Agricultural University (Varma, 2018). Pongamia oil was obtained from Tamil Nadu Agricultural University, Coimbatore to make pongamia oil soap required for the experiment. To prepare pongamia oil soap, 55g of caustic soda was mixed with 100ml of distilled water and left undisturbed for 4 hours. The solution blended scrupulously into the solution made up of 55g of soap stone power and one litre of pongamia oil. The final pongamia oil soap solution kept for solidification. The pongamia oil soap solution pH was determined (10.5) in Soil Science and Agricultural Chemistry lab, College of Agriculture, Padannakkad and saponification value was 194mg KOH/g of oil. Neem oil soap 0.6 per cent and standard check (Thiamethoxam) were also included as treatments to compare the results of pongamia oil soap.

Experiments were performed with the KAU released brinjal variety "Surya" under Randomized Block Design with 8 treatments (Table 1) and three replicates at Instructional Farm II, Karuvachery, College of Agriculture, Padannakkad. Eggplant seeds were sown in pro trays and 30 days old seedlings transplanted in a plot of size 3.4 X 2.8m$^2$ each with the spacing of 60 X 60cm. Pongamia oil soap 3, 2, 1 and 0.6 per cent spray solution were prepared by using pongamia oil soap 30, 20, 10 and 6g pongamia oil soap respectively and knapsack sprayer was used for spraying. Five representative plants among 12 plants were selected and tagged for recording observations. The observations were documented one day before, 1, 3, 5, 7 and 14 days after treatment application. The nymphs and adult red spider mites (RSM) and its natural enemies (predatory gall midge and rove beetle) were counted from the 2 cm$^2$ leaf area of three leaves (one each from top, middle and lower) from represented plants. To calculate the per cent reduction in red spider mite population, Henderson and Tilton formula was used (Herderson and Tilton, 1955).

$$\text{Per cent reduction} = \{1 - \left(\frac{\text{n in C before treatment x n in T after treatment}}{\text{n in C after treatment x n in T before treatment}}\right)\} \times 100$$

The data on mites and its natural enemy's population count were analyzed after square root transformation by analysis of variance (ANOVA). Web Agri Stat Package (WASP) software was used to analyze the data on population count of mites and its enemies.

## RESULTS AND DISCUSSION

**Red spider mite population:**

RSM population, ranging from 43.67 to 54.53 per 3 leaves recorded one day before the treatments, showed no significant difference among treatments. Pongamia oil soap 3per cent had significantly lower mites (5.53 RSM/3 leaves), comparable to 2 per cent pongamia oil soap on a day after spray application. The soap solution recorded the maximum mite population (91.40 RSM/3 leaves), comparable to the control plot (48.53 RSM/3 leaves). Pongamia oil soap at 1 and 0.6 per cent showed statistically equivalent results in reducing - mite populations (11.53 and 12.67 RSM/3 leaves respectively). Neem oil soap at 0.6 per cent (20.20 RSM/3 sheets) were found to be equivalent to thiamethoxam 25 WG (33.73 RSM/3 sheets) and both were comparable to pongamia soap at 0.6 and 1 percent.

Three days after spray, pongamia oil soap (3%) had the lowest mite population – (16.33 RSM/3 leaves), followed by pongamia oil soap at 2, 1, 0.6 per cent and neem oil soap (20.47, 21.07, 21.93 and 27.27 RSM/3 leaves respectively) and they were on par with each other. The 0.5 per cent soap solution recorded the highest mite count (55.07 RSM/3 leaves), comparable to the control (50.73

Table 1. Average population density of red spider mites, *Tetranychus urticae*

| Treatments | Number of mites per 3 $cm^2$ area of three leaves * | | | | | |
|---|---|---|---|---|---|---|
| | Second application | | | | | |
| | 1 DBS | 1 DAS | 3 DAS | 5 DAS | 7 DAS | 14 DAS |
| Thiamethoxam 25 WG 2g $10L^{-1}$ | 54.53 (6.93) | 33.73 (5.74)$^{bc}$ | 35.13 (5.69)$^{ab}$ | 61.53 (7.83)$^{a}$ | 74.47 (8.61)$^{ab}$ | 85.33 (9.22)$^{a}$ |
| Pongamia oil soap 3% | 43.67 (6.59) | 5.53 (2.23)$^{d}$ | 16.33 (4.02)$^{b}$ | 24.40 (4.93)$^{c}$ | 35.13 (5.92)$^{f}$ | 47.67 (6.92)$^{d}$ |
| Pongamia oil soap 2% | 50.47 (7.09) | 7.87 (2.78)$^{d}$ | 20.47 (4.50)$^{b}$ | 34.87 (5.89)$^{bc}$ | 36.60 (6.03)$^{ef}$ | 55.73 (7.43)$^{cd}$ |
| Pongamia oil soap 1% | 46.20 (6.70) | 11.53 (3.34)$^{cd}$ | 21.07 (4.55)$^{b}$ | 35.67 (5.96)$^{b}$ | 41.67 (6.43)$^{def}$ | 62.47 (7.93)$^{bcd}$ |
| Pongamia oil soap 0.6% | 51.00 (7.11) | 12.67 (3.45)$^{cd}$ | 21.93 (4.63)$^{b}$ | 38.93 (6.22)$^{b}$ | 50.60 (7.10)$^{cde}$ | 64.93 (8.07)$^{abcd}$ |
| Neem oil soap 0.6% | 47.13 (6.85) | 20.20 (4.37)$^{bcd}$ | 27.27 (5.16)$^{b}$ | 37.93 (6.13)$^{b}$ | 53.93 (7.33)$^{cd}$ | 65.47 (8.09)$^{abc}$ |
| Soap solution 0.5% | 67.00 (7.70) | 91.40 (9.10)$^{a}$ | 55.07 (7.30)$^{a}$ | 76.67 (8.74)$^{a}$ | 85.13 (9.18)$^{a}$ | 73.20 (8.58)$^{ab}$ |
| Control | 47.47 (6.89) | 48.53 (6.96)$^{ab}$ | 50.73 (7.09)$^{a}$ | 64.73 (8.02)$^{a}$ | 59.40 (7.70)$^{bc}$ | 74.73 (8.67)$^{ab}$ |
| C.D. (P=0.05) | NS | 2.60 | 1.86 | 1.03 | 1.17 | 1.16 |

* Mean of five observations; Means followed by similar alphabets don't differ significantly by DMRT at 5%; Figures in parentheses denotes square root transformed values

RSM/3 leaves). Plants treated with thiamethoxam 25 WG (35.13 RSM/3 leaves) were comparable to all other treatments tested. Observations made 5 days after treatment pongamia soap (3%) showed least 24.40 RSM/3 leaves, followed by pongamia soap (2 and 1%), neem oil soap and pongamia soap (0.6%) with 34.87, 35.67, 37.93 and 38.93 RSM/3 leaves respectively. The soap solution (0.5%) recorded the highest mite count of 76.67 RSM/3 leaves. This was comparable to controls (64.73 RSM/3 leaves) and thiamethoxam 25 WG (61.53 RSM/3 leaves).

Mite populations were lowest on plants treated with pongamia soap @ 3 per cent (35.13 RSM/3 leaves) on day 7 after treatment, compared to pongamia soap @ 2 and 1 percent with 36.60 and 41.67 RSM respectively. It was 50.60 RSM/3 leaves in pongamia soap (0.6%) which was on par with neem oil soap 0.6 per cent (53.93 RSM/3 leaves), and pongamia soap 1 and 2 per cent. The maximum population was observed in 0.5 per cent soap solution (85.13 RSM/3 leaves), followed by thiamethoxam 25 WG (74.47 RSM/3 leaves), and the control (59.40 RSM/3 leaves).

Increased mite populations were observed for all treatments. Pongamia soap (3%) recorded 47.67 RSM/3 leaves after 14 days of spraying - followed by pongamia oil soap 2 per cent (55.73 RSM/3

leaves), 1 per cent (62.47 RSM/3 leaves) and 0.6 per cent (64.93 RSM/3 cards) pongamia soap. Neem oil soap 0.6 per cent (65.47 RSM/3 leaves) was comparable to soap solution 0.5 per cent (73.20 RSM/3 leaves), pongamia oil soap 0.6 and 1 per cent, control (74.73 RSM/3 leaves) and thiamethoxam treated plot showed maximum mite population (Table 1).

**Percentage reduction in spider mite population:**

Pongamia oil soap 3, 2, and 1 percent were statistically equivalent, reducing mite populations by 86.50, 84.57, and 79.27 per cent, respectively whereas soap solution 0.5 per cent (0.17%) and the insecticide thiamethoxam 25 WG (19.60%) were comparable with control (0.00%) in population decline. Pongamia oil soap 0.6 per cent with a population reduction of 76.40 per cent was equivalent to 1, 2, and 3 per cent pongamia oil soap at 1 day post-treatment. Three days after spray application, a maximum population reduction was recorded in pongamia soap 3 per cent which was on par with pongamia oil soap 2, 1 and 0.6 per cent with 61.17, 57.90, 55.10 and 53.93 per cent decrease in population of mites respectively. At 0.5 per cent soap solution, it was comparable to the control (0.00%) and thiamethoxam 25 WG (32.43%) with only a 16.00 per cent reduction. Neem oil soap 0.6 per cent reduced the population by 45.73 per cent among botanicals, comparable with pongamia soap at 3, 2, 1 and 0.6 per cent.

The pongamia oil soap 3 per cent was the highest among all treatments in population reduction (55.67%), while the soap solution increased mite populations with a negative sign (-23.3%). This was consistent with thiamethoxam 25 WG (5.33%) and comparable to the control (0.00%) at 5 days of application. Pongamia soap @ 2 and 1 per cent, and neem oil soap 0.6 per cent were statistically similar in population decline, recording 44.00, 41.40, and 38.00 3 per cent respectively. After 7 days of application, pongamia soap @ 3 and 2 per cent showed reduction in mite population (42.00 and 40.33%, respectively). Pongamia oil soap @ 1 and 0.6 per cent showed statistically similar results in reducing mite population (18.00 and 17.02% respectively). However, the soap solution, thiamethoxam, and untreated plots showed no reduction in population and recorded negative results (of -47.00, -35.00, and 0.00%, respectively).

On the 14th day of application, population reduction was statistically non-significant among pongamia soap 3, 2, 1, 0.6 per cent and neem oil soap 0.6 per cent treated plots (31.33, 19.00, 18.67, 17.67 and 16.33% respectively). However, thiamethoxam 25 WG and soap solution 0.5% failed to reduce mite numbers, indicated by negative signs of -32 and -4.3 per cent, both comparable to the control plot (Table 2).

**Relative abundance of mite predators:**

Data on a day before spray application revealed that the mite predator's numbers didn't differ significantly among different treatments and it was at a range of 1.33 to 4.00/5 plants. Mite predator's abundance did not change significantly 1 day after spray application it was at a range of 1.33 to 4.33/ 5 plants. Three days after spray, predator populations were statistically non-significant and evenly distributed (1.67 to 5.67). After 5 days of spray also, there was no significant difference in – predator (1.67 to 7.67/ 5 plants). Observations on 7 DAS revealed that the 0.5 per cent soap solution treatment recorded more predator population of 11.00/5 plants, and significantly different from the other treatments. Pongamia oilseed soap 0.6 per cent recorded 9.33/5 plants and was statistically equivalent to 0.5 per cent soap solution. Pongamia soap 2 per cent and 0.6 per cent neem soap treatments were statistically similar with comparable results to control - ( 4.00, 4.00 and 4.33/ 5 plants, respectively), but the standard test and 3 per cent pongamia soap showed the lowest population- (0.33 and 2.33/5 plants respectively).

Abundance of mite predators was relatively high in all treatments on 14 DAS. Soap solution (0.5%) recorded highest at 35.67/5 plants which was comparable to pongamia oil soap at 0.6, 1 and 2 per cent with mite predator populations of 20.00,

Table 2. Percentage reduction in red spider mite's population during field evaluation

| Treatments | Percentage reduction in mites * | | | | |
|---|---|---|---|---|---|
| | Second application | | | | |
| | 1 DAS | 3 DAS | 5 DAS | 7 DAS | 14 DAS |
| Thiamethoxam 25 WG 2g $10L^{-1}$ | 19.60 $^{c}$ | 32.43 $^{bc}$ | 5.33 $^{c}$ | -35 $^{c}$ | -32 $^{b}$ |
| Pongamia oil soap 3% | 86.50 $^{a}$ | 61.17 $^{a}$ | 55.67 $^{a}$ | 42.00 $^{a}$ | 31.33 $^{a}$ |
| Pongamia oil soap 2% | 84.57 $^{a}$ | 57.90 $^{a}$ | 44.00 $^{ab}$ | 40.33 $^{a}$ | 19.00 $^{a}$ |
| Pongamia oil soap 1% | 79.27 $^{a}$ | 55.10 $^{a}$ | 41.40 $^{ab}$ | 18.00 $^{b}$ | 18.67 $^{a}$ |
| Pongamia oil soap 0.6% | 76.40 $^{ab}$ | 53.93 $^{a}$ | 37.07 $^{b}$ | 17.02 $^{b}$ | 17.67 $^{a}$ |
| Neem oil soap 0.6% | 57.13 $^{b}$ | 45.73 $^{ab}$ | 38.00 $^{ab}$ | 6.33 $^{bc}$ | 16.33 $^{a}$ |
| Soap solution 0.5% | 0.17 $^{c}$ | 16.00 $^{cd}$ | -23.3 $^{c}$ | -47.00 $^{c}$ | -4.3 $^{b}$ |
| Control | 0.00 $^{c}$ | 0.00 $^{d}$ | 0.00 $^{c}$ | 0.00 $^{c}$ | 0.00 $^{b}$ |
| C.D. (P=0.05) | 21.03 | 21.04 | 17.78 | 13.14 | 15.96 |

* Mean of observations of five plants; Means followed by similar letters are not significantly different by DMRT at 5%; DAS- Days After Spray; NS – Non-Significant

19.67 and 18.00/5 plants respectively. All remaining treatments were comparable to control plots (9.33/ 5 plants) containing neem oil soap @ 0.6 per cent, pongamia oil soap @ 3 per cent, and standard checks, each with a mite predator population of 12.33, 11.33, and 8.00/ 5 plants (Table 3).

Overall, pongamia soap solutions (@ 0.6, 1, 2, and 3%) were superior to control in reducing the RSM population. The greatest reduction was observed immediate day after spraying, and all treatments except soap solution remained effective up to 5 days after spraying, after which they declined. This observation is consistent with the acaricidal effect of pongamia oil against the horseshoe mite (*Tetranychus ludeni*). Among the various sprayed chemical treatments, 0.6ml $l^{-1}$ Abamectin 1.8 EC showed the greatest reduction in mite population (70.32%) on 14$^{th}$ day of spraying, followed by spiromesifen and fenpyroximate while pongamia oil at 2 ml/l decreased by 49.58% on the fourteenth day (Raina, 2016). Among botanicals, tulsi leaf extract (5 and 10%), neem oil (3 and 5%) and notchi leaf extract (5 and 10%) recorded superior results followed by 63.53 and 62.98 per cent egg reduction were recorded by pongamia oil soap at 3 and 5 per cent respectively (Raghavendra *et al*., 2017).

The results obtained in the current study show that the miticidal effect of pongamia soap 0.6 per cent is better than that of 0.6 per cent neem oil soap. This result is in consistent with the study reporting, among the plants evaluated, pongamia oil was clearly the superior treatment, showing high mortality with a low $LC_{50}$ value of 0.008 per cent whereas neem oil recorded higher $LC_{50}$ value of 0.230 per cent at 24 h after treatment (Islam *et al*., 2017). In a another study same results was found with abamectin reducing the population of red spider mite (*Tetranychus urticae*) on okra by 74.64 per cent, recording the highest efficacy (Singh *et al*., 2018).

The nirgundi and pongamia oils were given better remedies than neem oil as evidenced by the minimum LC values of 78.40 and 194.20ppm respectively, and neem oil was 1469.88ppm against tea mite *Oligonychus coffeae* (Handique *et al*.,

2018). A reduction of up to 32.43 per cent was recorded on the third day after spraying, and from the fifth day onwards the effect began to decline, indicating an increase in the number of mites on the seventh and fourteenth day. In a study, thiamethoxam, clothianidin, and imidacloprid on cotton, corn, and tomatoes each showed increases in spider mite populations, and noted a 30 per cent increase in spider mite populations on cotton plants treated with thiamethoxam at the end of the study while in the current study are 32 per cent more mite numbers at 14DAS (Szczepaniec *et al*., 2013).

Data on rove beetle, *Oligota* and predatory gall midge, *Feltiella acarisuga* show that pongamia oil soap treated plants recorded higher population compared to even control plot. Soap solution 0.5 per cent recorded highest of 35.67/5 plants, followed by pongamia oil soap 0.6, 1 and 2 per cent. All remaining treatments were comparable to control plots (9.33/5 plants) containing neem oil soap @ 0.6 per cent, pongamia oil soap @ 3 per cent, and standard checks, each with a mite predator population of 12.33, 11.33, and 8.00/5 plants on 14$^{th}$ day after spray. As no previous studies on the

Table 3. Relative abundance of mite predators during field evaluation

| Treatments | Number of mite predators per 3 cm$^2$ area of three leaves* | | | | | |
|---|---|---|---|---|---|---|
| | Second application | | | | | |
| | 1 DBS | 1 DAS | 3 DAS | 5 DAS | 7 DAS | 14 DAS |
| Thiamethoxam 25 WG 2g 10L$^{-1}$ | 1.67 (1.46) | 1.67 (1.24) | 1.67 (1.35) | 1.67 (1.39) | 0.33 (1.68) $^{d}$ | 8.00 (2.67) $^{b}$ |
| Pongamia oil soap 3% | 1.67 (1.46) | 2.00 (1.41) | 1.67 (1.46) | 2.67 (1.76) | 2.33 (1.68) $^{cd}$ | 11.33 (3.09) $^{b}$ |
| Pongamia oil soap 2% | 2.33 (1.68) | 2.33 (1.52) | 2.00 (1.56) | 2.67 (1.77) | 4.00 (2.12) $^{c}$ | 18.00 (4.15) $^{ab}$ |
| Pongamia oil soap 1% | 2.00 (1.56) | 2.67 (1.63) | 3.00 (1.86) | 4.67 (2.13) | 8.33 (2.96) $^{b}$ | 19.67 (4.32) $^{ab}$ |
| Pongamia oil soap 0.6% | 2.00 (1.56) | 3.00 (1.71) | 3.33 (1.95) | 6.67 (2.64) | 9.33 (3.13) $^{ab}$ | 20.00 (4.42) $^{ab}$ |
| Neem oil soap 0.6% | 1.33 (1.27) | 1.33 (1.14) | 2.33 (1.68) | 1.67 (1.25) | 4.00 (2.09) $^{c}$ | 12.33 (3.50) $^{b}$ |
| Soap solution 0.5% | 4.00 (2.03) | 4.33 (1.94) | 5.67 (2.43) | 7.67 (2.81) | 11.00 (3.38) $^{a}$ | 35.67 (5.92) $^{a}$ |
| Control | 4.00 (2.09) | 2.67 (1.61) | 2.67 (1.74) | 6.00 (2.41) | 4.33 (2.18) $^{c}$ | 9.33 (3.05) $^{b}$ |
| C.D. (P=0.05) | NS | NS | NS | NS | 0.50 | 1.89 |

• Means followed by similar alphabets do not differ significantly @ 0.05 DMRT; Figures in parentheses denotes square root transformed values; DAS- Days After Spray; NS – Non-Significant

effects of pongamia treatment on predatory mites were available in the literature, the results on predatory mite populations are compared with neem products. Adverse effects of applying phytopesticides to the predatory mite *Amblyseius andersoni* were reported (Castagnoli *et al*., 2002). Eggs were unaffected by Biopyrene Plus (Pyrethrin 8 EC) (92.31% hatching) and Neemazal 10 EC (98.73% hatching) after treatment. Only 14.01 per cent toxicity was recorded on females by neemazal treatment whereas others showed 100 per cent toxicity. Survival percentage of protonymphs by neemazal treatment was 51.33 per cent and death was Zero.

It's been observed that the overall toxic effects of thiamethoxam were more than 90 per cent to *Phytoseiurus persimilis* by all different routes of exposure. However, the local exposure could result in low mortality and residual exposure (Pozzebon *et al*., 2011). The present study is in accordance with the findings in which the neonicotinoids are mildly toxic to *Neoseiulus fallacis* (Jamil *et al*., 2016), and neonicotinoids are moderately to highly toxic to *Galendromus occidentalis* and *G. fallacis* (Bostanian *et al*., 2009). It may be concluded pongamia oil soap showed fair control effect on red spider mites, in brinjal better than neonicotinoid thiamethoxam. At the same time, pongamia soap at different concentration showed no negative effects on mite predators hence it is absolutely safe to use against brinjal pests.

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# Eri silkworm pupae as fish meal replacement in common carp (*Cyprinus carpio* L.) feed in indoor aquaria

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**ABSTRACT:** Eri silkworm (*Samia ricini* D.) pupae based fish feed, for common carp (*Cyrinus carpio* L.) advanced fry, in indoor aquaria was evaluated with eight different diets. Eri silkworm pupae meal (ESWPM) based fish feeds influenced the growth of common carp significantly. Fortnightly growth measurements of the carp in eight treatments showed maximum in ESWPM alone and in ESWPM 100 per cent treatments. The proximate nutrient and amino acid composition varied in the fish feed diets. ESWPM based fish feed recorded significant impact on the carp with reference to its weight gain, specific growth rate, feed conversion ratio and relative growth rate. The technology got patent.


**KEYWORDS:** By-product utilization, *Samia ricini,* pupae, *Cyprinus carpio,* feed

Fish meal has long been a significant source of protein in the fish feed industry. Furthermore, wild fish catches have been progressively dropping due to aquaculture's heavy reliance on them as a feed source for farmed fish (FAO, 2014). Aquaculture feed costs make up between 40 and 70 percent of the price of the fish produced (Wilson, 2002; Rana *et al.,* 2009). Due to the shortage of fish meal, other protein sources with comparable nutritional characteristics have been thoroughly researched (Daniel, 2018). Studies on insect protein as a partial or complete substitute for the fish meal are attempted (Van Huis, 2013). Antifungal and antibacterial peptides in insect meal may also help the meal's shelf life.

Some conflicting opinions also arise about chitin-containing insect-based fish feed, which reduces nutrient absorption and digestibility and interferes with the fish's normal physiological functions. However, the three enzymes required for chitin digestion: chitinase, chitobiose, and lysozyme (Lindsay *et al.,* 1984; Fines and Holt, 2010), are present in both carnivorous and omnivorous fish species. Further, Aquatic organisms' immune systems are modulated by chitin, its derivatives, and active substances found in insect exoskeletons, such as antimicrobial peptides (AMPs). Chitin intake in moderate proportion can enhance fish gut health, immunity, and resistance to infectious diseases (Hoffman *et al.,* 1997; Kim and Rajapakse, 2005; Lin *et al.,* 2012; Harikrishnan *et al.,* 2012). Chitin is responsible for some of the immunostimulatory

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effects of insect meal (Su *et al.,* 2017; Li *et al.*, 2017; Gasco *et al.,* 2018; Henry *et al.,* 2018).

According to Chakrabarthy *et al.* (1973), silkworm pupae are a better meal for Indian major carps than mustard oil cake and rice bran. Among silkworms, the two popular types are mulberry silk worm (MSW), feeding on mulberry, and eri silk worm (ESW), feeding on castor. Using the under-utilized by-products, MSW and ESW pupae, containing nearly 54 and 62 per cent protein, respectively, as an alternative protein source provides ample scope (Zegeye, 2020; Altomere *et al.,* 2020). Moreover, the enormous potential for using ESW pupae as a feed resource is made possible by their composition, which includes 26.21 per cent lipid, 8 per cent moisture content, 56.83 per cent Poly Unsaturated Fatty Acids (PUFA), high methionine and lysine content, low omega-6/omega-3 fatty acid, and sodium: potassium ratio (Mrinal Ray and Gangopadhyay, 2021).

One of the most popular and commercially significant freshwater fish is the common carp, *Cyprinus carpio*, which accounts for 11 per cent of global freshwater aquaculture production (FAO, 2014). Global production of common carp accounts 3.4 per cent of the world's fish production. During its transitions from the advanced fry to the fingerlings stage, insects become one of its main sources of nutrition (Aquaculture feed and fertilizer resource information system, FAO, 2014). The current study aimed to determine ESW pupae's suitability as a protein supplement replacing fish meal in common carp feed.

## MATERIALS AND METHODS

The ESW culture was maintained in the Department of Entomology, Faculty of Agriculture, Annamalai University, Tamil Nadu, India. On the fifth day of spinning, cocoons were cut open, pupae taken out, dried in a hot air oven (Technico, TLPPL 131) at 60ºC for 12 hours, and made into powder. Various ratios of feed ingredients, *viz.,* ESW pupae powder, fish meal, groundnut cake, rice bran, fish oil, tapioca flour, and vitamin and mineral premix (Table 1), were ground and sieved through a 250-micron mesh. The ingredients were combined with enough water, mixed into a soft dough, cooked under pressure for 15 minutes, cooled, and then pushed through a manual noodle's maker (with a 1mm diameter sieve plate) to obtain noodle-like threads. They were then baked in a hot air oven at 60°C for six hours to lower the moisture content to 8 per cent, broken and sieved to obtain the required pellet size (0.5 - 1.5mm), depending on the age of the fish. The prepared isocaloric and isonitrogenous ESW pupae-based fish feeds were packed in airtight plastic containers separately and stored at room temperature.

*Cyprinus carpio* advanced fries with a mean weight range from 0.300 – 0.450g were purchased from a commercial fish farm (M/s Shakthi fish farm). They were acclimatized for about two weeks with a continuous oxygen supply using an aquarium air pump (Sebo, AP 500) and commercial fish feed (Vrudhi Plus, Godrej Agrovet Limited, Mumbai). Then they were randomly stocked into an experimental glass tank (60cm x 45cm x 45cm). Feed was given daily once with a ratio of five per cent fish body weight. The faecal matter was siphoned out and a 75 per cent water change was done at weekly intervals. The experimental design was planned as a Completely Randomised Design (CRD) consisting of eight treatments, three replications, and ten fish per replication. Growth indices like Weight Gain (g), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Relative Growth Rate (RGR), Survival Rate (%) and Fish In: Fish out (FIFO) (%) were calculated for ten weeks. Experimental feeds' proximate nutrient composition and essential amino acid composition analysis using three samples from each treatment were carried out following Sadasivam and Manickam (2005) and Bandlamori *et al.* (2012) protocol, respectively. The data were subjected to one-way ANOVA and analysed using SPSS Statistical package version 16.

**Formulas for growth indices:**

Weight Gain (g) = Final wet weight – Initial wet weight

Table 1. Composition of experimental diets (per 100 g) in the treatments

| Feed Composition | ESWPM 20 % | ESWPM 40 % | ESWPM 60 % | ESWPM 80 % | ESWPM 100 % | ESWPM alone | Control | Positive* Control |
|---|---|---|---|---|---|---|---|---|
| Fish meal (g) | 24 | 18 | 12 | 6 | 0 | - | 30 | - |
| Eri Silkworm pupae powder (g) | 6 | 12 | 18 | 24 | 30 | 62 | 0 | - |
| Groundnut cake powder (g) | 20 | 20 | 20 | 20 | 20 | - | 20 | - |
| Rice husk (g) | 37 | 37 | 37 | 37 | 37 | - | 37 | - |
| Fish oil (ml) | 2 | 2 | 2 | 2 | 2 | - | 2 | - |
| Tapioca flour (g) | 10 | 10 | 10 | 10 | 10 | - | 10 | - |
| Vitamin and Mineral premix (g) | 1 | 1 | 1 | 1 | 1 | - | 1 | - |
| Bentonite | - | - | - | - | - | 3 | - | - |
| Cellulose | - | - | - | - | - | 35 | - | - |

ESWPM - Erisilk worm pupae meal; *Commercial fish feed used as such

$$\text{Specific Growth Rate (SGR)} = \frac{(\text{Ln(Wt)} - \text{Ln(W0)}}{\text{t(d)}} \text{X } 100$$

W0[g]= the weight in grams at the beginning of the period

Wt [g]= the weight in grams at the end of the period

t[d]= period, expressed in number of days

Ln = natural logarithm

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed Fed}}{\text{Wet weight gain}}$$

$$\text{Relative Growth Rate (RGR) (\%)} = \frac{\text{Final Weight-Initial Weight}}{\text{Initial Weight}}$$

$$\text{Survival (\%)} = \frac{\text{Number of live fishes at end of the experiment}}{\text{Total numbers of fishes Stocked}} \text{ X } 100$$

Fish In: Fish Out (FIFO) (%) = FCR X (% Fish meal + Fish oil in feed)

## RESULTS AND DISCUSSION

### Impact of eri silkworm pupae-based fish feed on common carp's growth performance

It was found that the ESW pupae-based fish feed significantly influenced common carp's growth indices. This performance of ESW pupae meal (ESWPM) at 20 and 40 per cent, recorded poor fortnight weight gains (Table 3). Such poor performance resulted due to the poor protein content of the feed fed (as both treatments contain more proportion of fish meal over ESWPM than other treatments). Nandeesha *et al.* (1990) reported such a reduced growth in common carp when fed with a diet containing 10 and 20 per cent non-defatted pupae meal. In contradiction to poor performance of ESWPM treatment, Jayaram and Shetty (1980a) reported better growth in 30 per cent defatted pupae meal fed catla and common carp.

During the first fortnight measurement, the maximum growth was recorded in ESWPM 100 per cent (5.1g) and ESWPM alone (5g) both treatments recorded on par with each other. Positive

Table 2. Proximate nutrient and essential amino acid composition of experimental feeds in the Treatments

| Composition | ESWPM (20 %) | ESWPM (40 %) | ESWPM (60 %) | ESWPM (80 %) | ESWPM (100 %) | ESWPM alone | Control | Positive Control (Commercial fish feed) |
|---|---|---|---|---|---|---|---|---|
| Crude protein (%) | 38.1 | 38.3 | 38.0 | 38.4 | 38.5 | 38.5 | 38.4 | 38 |
| Crude lipid (%) | 8.3 | 8.2 | 8.1 | 8.0 | 8.6 | 9.4 | 8.2 | 8.5 |
| Crude fibre (%) | 1.1 | 1.3 | 1.1 | 1.2 | 1.1 | 1.2 | 1.2 | 0.8 |
| Metabolizable energy ($MJKg^{-1}$) | 17.5 | 17.2 | 17.4 | 17.3 | 17.6 | 18 | 17.3 | 17.0 |
| Iron (mg 100 $g^{-1}$) | 13.1 | 13.0 | 13.3 | 13.5 | 13.8 | 13.5 | 13.7 | 12.9 |
| Zinc (mg 100 $g^{-1}$) | 20.5 | 21.0 | 21.8 | 20.9 | 21.3 | 21.9 | 21.2 | 20.6 |
| Calcium (mg 100 $g^{-1}$) | 1.4 | 1.5 | 1.5 | 1.9 | 2.0 | 2.1 | 1.4 | 1.3 |
| Phosphorus (mg 100$g^{-1}$) | 1.1 | 1.2 | 1.3 | 1.2 | 1.4 | 1.5 | 1.4 | 1.5 |
| Methionine (g 100 $g^{-1}$) | 1.1 | 1.1 | 1.4 | 1.3 | 1.5 | 1.6 | 1.5 | 1.4 |
| Cystine (g 100 $g^{-1}$) | 1.3 | 1.3 | 1.2 | 1.4 | 1.5 | 1.6 | 1.9 | 1.6 |
| Lysine (g 100 $g^{-1}$) | 2.7 | 2.5 | 3.3 | 3.0 | 3.3 | 3.7 | 4.0 | 3.2 |

control and ESWPM 80 per cent recorded next best weight gains. However, the trend was little bit changed in the second fortnight, ESWPM alone recorded higher value (12.6 g) followed by ESWPM 100 per cent (12.1 g). In third fortnight measurement, ESWPM 100 per cent and ESWPM alone both treatments were on par with each other. It was followed by positive control and ESWPM 80 per cent recorded on par with each other. Control treatment and ESWPM 60 per cent also were on par with each other. In fourth fortnight interval, ESWPM 80 per cent recorded weight a head than positive control. In last fortnight measurement (fifth fortnight interval), trend recorded in third fortnight interval evidenced. Irrespective of all the fortnight interval measurements ESWPM 40 and 20 per cent records the poor growth performance (Table 3). Weight gain (%) in experimental feed-fed fish over positive control represent the significant performance of ESWPM alone (23.57 % increase over positive control) and ESWPM 100 per cent (20.29 % increase over positive control) among other treatments.

Rangacharyulu *et al.* (2003) reported a 13 per cent higher weight gain when fish meal was entirely replaced with silkworm pupae silage in the Indian major carp's diet. The reason behind the enhanced performance is due to the presence of hydrolysed protein in ensiled silkworm pupae as compared to the complex proteins in fish meal (Manikandavelu *et al.,* 1992; Anon, 1999). Wan *et al.* (2017) reported that silkworm pupae meal is an attractive and sustainable functional feed component in carp diet with enhanced growth performance. Better protein and fat digestibility of carp diet containing silkworm pupae meal than the fish meal was reported by Nandeesha *et al.* (1990) were in line with the present study findings. However, Nandeesha *et al.* (1989 a, b) and Ji *et al.* (2015) reported least growth performance, impaired antioxidant enzyme status, decreased digestive function, and unfavourable changes in hepatic and intestinal morphology in fish fed with defatted silkworm pupae entirely or in combination with fish meal at different proportions. Further, Nandeesha *et al.* (2000) found 50 per cent silkworm pupae

Table 3. Weekly growth performance (weight gain) of fish fed on experimental feeds (g) at fortnightly intervals

| Treatments | Initial | 1st | 2nd | 3rd | 4th | 5th |
|---|---|---|---|---|---|---|
| ESWPM 20 % | 0.358 (0.598)[b] | 2.4 (1.549)[d] | 4.8 (2.191)[f] | 9 (3.000)[e] | 14.4 (3.860)[e] | 19.8 (4.505)[e] |
| ESWPM 40 % | 0.333 (0.576)[b] | 2.4 (1.549)[d] | 5.7 (2.387)[e] | 10.2 (3.193)[d] | 15.2 (3.964)[e] | 21.3 (4.669)[d] |
| ESWPM 60 % | 0.337 (0.580)[b] | 3.5 (1.871)[c] | 8.8 (2.966)[d] | 14.3 (3.781)[c] | 18.7 (4.382)[d] | 28.2 (5.357)[c] |
| ESWPM 80 % | 0.333 (0.576)[b] | 3.9 (1.975)[b] | 9.8 (3.130)[c] | 16.2 (4.025)[b] | 22.5 (4.796)[b] | 31.1 (5.621)[b] |
| ESWPM 100 % | 0.365 (0.604)[a] | 5.1 (2.258)[a] | 12.1 (3.480)[b] | 19.9 (4.462)[a] | 27.2 (5.263)[a] | 36.2 (6.060)[a] |
| ESWPM alone | 0.424 (0.652)[a] | 5 (2.236)[a] | 12.6 (3.559)[a] | 20.5 (4.528)[a] | 28 (5.338)[a] | 37.1 (6.132)[a] |
| Control | 0.404 (0.635)[a] | 3.5 (1.871)[c] | 8.6 (2.932)[d] | 14.2 (3.768)[c] | 18.6 (4.370)[d] | 27.9 (5.329)[c] |
| Positive Control (Commercial feed) | 0.414 (0.643)[a] | 3.8 (1.949)[b] | 9.5 (3.082)[c] | 15.6 (3.949)[b] | 21.3 (4.669)[c] | 30.2 (5.543)[b] |
| SEd | 0.008 | 0.023 | 0.036 | 0.047 | 0.054 | 0.065 |
| C.D (0.05) | 0.016 | 0.046 | 0.072 | 0.094 | 0.109 | 0.130 |

Mean of three replications; Values within parentheses are square root transformed

inclusion diet were optimal to maintain growth performance and meat quality in common carp, contradicting the present finding of optimal growth performance in inclusion diets containing ESWPM above 60 per cent. As the fish meal incorporated might be contaminated, the resulting protein quality might also be inferior leading to conflicting results**.** Moreover, eri silkworm pupae's nutritive profile is completely different from mulberry silkworm pupae and fishmeal.

Poor weight gain recorded in fish fed with low proportion of ESW pupae powder might be due to amino acid profile imbalance. This was corroborated by the Kaushik and Seiliez (2010) and Wan *et al.* (2017). This was evidenced by the differences in amino acid profile of different feeds. The proximate nutrient and aminoacid composition different treatment fish feeds indicated variation (Table 2).

Similarly, Hora and Pillay (1962) found higher silkworm pupae incorporation led to offensive odour. Such odour related issues were observed in the present study only in the treatment ESWPM alone. Remaining ESW pupae-based fish feeds was in tune with the findings of Jayaram and Shetty (1980b) who reported no adverse flavour.

There was significant impact of eri silkworm pupae-based fish feed on common carp's growth indices (Table 4). Among the experimental feeds, fish fed with ESWPM alone recorded the highest weight gain of 36.67g followed by fish fed with ESWPM 100 per cent (35.86 g) and positive control (29.81g). Treatment ESWPM 60, 40 and 20 percent recorded least weight gains. Wan *et al.* (2017) studied the

Table 4. Growth indices of different experimental feeds fed common carp

| Treatments | Weight gain | Specific Growth Rate (SGR) | Feed Conversion Ratio (FCR) | Relative Growth Rate (RGR) | Survival Rate (%) | Fish In: Fish Out (FI:FO) (%) |
|---|---|---|---|---|---|---|
| ESWPM 20 % | 19.44 | 2.79 | 2.5 | 54.3 | 100 | 20 |
| ESWPM 40 % | 20.96 | 2.79 | 2.3 | 62.94 | 100 | 32.2 |
| ESWPM 60 % | 27.86 | 3.21 | 1.7 | 82.67 | 100 | 34 |
| ESWPM 80 % | 30.76 | 3.34 | 1.6 | 86.48 | 100 | 41.6 |
| ESWPM 100 % | 35.86 | 3.68 | 1.3 | 92.37 | 100 | - |
| ESWPM alone | 36.67 | 3.93 | 1.3 | 98.24 | 100 | - |
| Control | 27.49 | 3.46 | 1.8 | 68.04 | 100 | 57.6 |
| Positive Control (Commercial feed) | 29.81 | 3.61 | 1.6 | 72 | 100 | - |

effect of marine (Ragworm) and terrestrial invertebrate meals (Silkworm Pupae) in the diet of *C. carpio* and reported an increased weight gain of 12 per cent in diets containing fish meal + silkworm pupae when compared with the reference control diet.

Maximum percent increase in fish weight per day indicated by the parameter, Specific Growth Rate (SGR) was recorded in ESWPM alone. On an average, it recorded 3.93 per cent daily weight gain. The ESWPM 100 per cent and positive control recorded 3.68 and 3.61 per cent daily weight gains, respectively (Table 4).

Least Feed Conversion Ratio (FCR) of 1.3 was obtained from ESWPM alone and ESWPM 100 per cent followed by ESWPM 80 per cent and positive control records 1.6. Poor FCR noticed in ESWPM 40 and 20 per cent, hence both this feed composition is not recommended for commercial pisciculture (Table 4).

Relative Growth Rate (RGR) was higher in ESWPM alone (98.24) followed by ESWPM 100 per cent (92.37). This denotes the significant better performance than other treatments. Least RGR values 62.94 and 54.3 were in ESWPM 40 and 20 per cent respectively. No negative impact on survival rate (%) was recorded in all the experimental diets fed fishes. Trend in FIFO was exact *i.e.,* the feed contains higher proportion of fish meal records higher values correctly.

From the present research, it was found that treatments, ESWPM alone and ESWPM replacing 100 per cent fish meal performed better than other experimental feeds. The technology got the patent from the Controller General of Patents, Designs & Trade marks, Chennai [No.136821 dt 18-12-2023]. However, the turbidity and the bad odour caused by ESWPM within few days after application need to be addressed.

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# Record of *Anoplocnemis phasianus* (Fabricius, 1781) (Hemiptera, Heteroptera, Coreidae) from Goa, India

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**ABSTRACT:** In the survey on diversity of Coreidae, *Anoplocnemis phasianus* (Fabricius, 1781) is recorded for the first time in the state of Goa. External morphology of *A. phasianus* is described with its present geographical distribution, taxonomic photo plate, host plants, and natural photographs of the nymphs and adults are provided. 

**KEY WORDS**: Survey, diversity, host plant, morphology

Coreidae, commonly known as squash bug or leaf-footed bug, are medium to large sized bugs with four jointed antennae; some species in this family are bright colored with a head that is narrower than the pronotum, a four-segmented beak, front wings with veins, and three-segmented tarsi; extended hind tibiae in some species form a leaf like appearance (Gupta *et al.*, 2012). Their repugnatory glands release an unpleasant odour (Moody, 1930) that are supporting the defensive mechanisms against the predator species. They feed on cucurbits like squash and pumpkin, but some of them are pests of different agricultural crops (Bonjour and Fargo, 1989; Bonjour *et al.,* 1990). While conducting a bug survey in Goa, nymphs and adults of *Anoplocnemis phasianus* (Fabricius) were observed on *Senna obtusifolia* (Linn.) and it was identified by using literature of British Fauna of India (Distant, 1902), this is the first record of this taxon in Goa. Young shoots, flowers, leaves, and stems of *S. obtusifolia* were found to be infested frequently.

## *Anoplocnemis phasianus* (Fabricius, 1781) (Plate 1: figs. 1-7; Plate 2: figs. 1-12)

*Lygaeus phasiana*, Fabricius 1781, *Spec. Ins*, 2: p 361

*Lygaeus grossipes*, Fabricius 1803. *Syst. Rhyng*., 2: p 205.

*Cerbus tumidipes*, Herrich- Shaeffer 1842, *Wanz. Ins*., 6: p.54.

*Mictis punctum*, affinis, bicolor. Westwood 1842, *in Hope Cat*, 2: p. 10.

*Anoplocnemis phasiana*: Distant 1902, *Fauna. Brit. Ind*., 1:p. 346.

**Specimens examined:**

Male, 13. viii. 2021, Verna (North Goa), elevation (15m), coordinates (15°21'36'' N; 73°55'44'' E), Coll. Ayesha Shetkar, deposited in ADKS College, Dodamarg; Male, 18. viii. 2021, Verna (North Goa), elevation (15m), coordinates (15°21' 36'' N; 73°55'44'' E), Coll. Aishwarya Naik, deposited in

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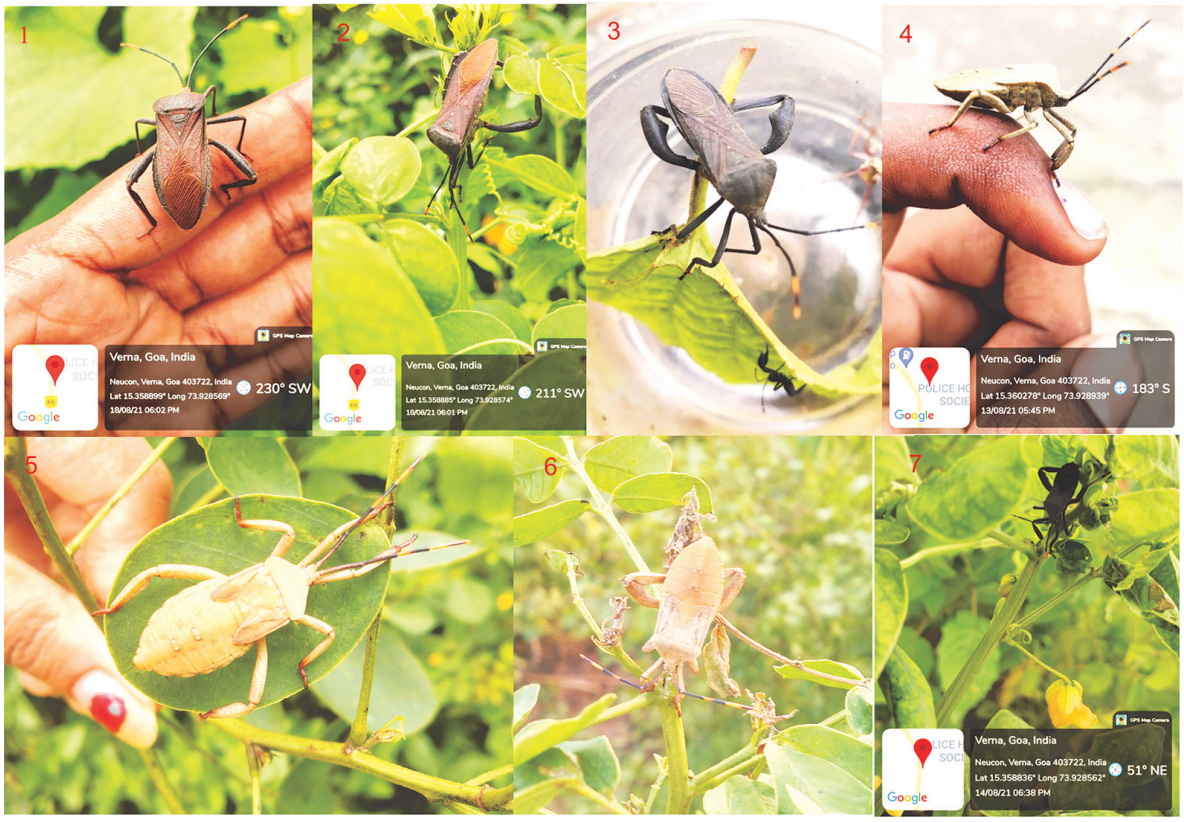


Plate 1: Figures 1-7. *Anoplocnemis phasiana* (Fabricius). 1. Dorsal view of female (brown colour); 2. Dorsal view of male (brown colour); 3. Dorsal view of male (black colour); 4. Fourth instar nymph, female; 5. Fourth instar nymph,female; 6 to7. Fifth instar nymphs on host plant.

ADKS College, Dodamarg; Female, 21. viii. 2022, Sal (North Goa), elevation (2m), coordinates (15°41'53'' N; 73°55'47'' E), Coll. Ayesha Shetkar; Female, 21. x. 2022, Kasarpal (North Goa), elevation (20 m), coordinates (15°38'44'' N; 73°56'26'' E), Coll. Parshuram Naik, deposited in ADKS College, Dodamarg. Host plant *Senna obtusifolia (*Linn.). Measurements (in mm). Male: total body length about 26.9 to 27.1 and Female: total body length about 24.8 to 25.

**Diagnostic characters: (Male)**

**Head:** Head dorsally black, longer than broad, pubescence, ventrally brownish to black; clypeus somewhat longer than paraclypei; antenniferous tubercle prominent and slightly overhanging the median lobe, widely separated; antennal segments black to brownish and setose; first three segments black; apical segment luteous at base and apex; antennal segments I stout and longer than segment II; segments I and IV slightly subequal; segment III smallest; segments II and III stout and slender; segment IV spindle shaped; eyes large and globose with dark brown colour, ocelli very close to eyes with light brown colour; buccula black and short, extending beyond antenniferous tubercle; rostrum short and black, extending beyond procoxae.

**Thorax:** Pronotum black, densely granular and setose, and sloping towards the head, length shorter than width; anterior lobe narrow and acute, separated by transverse sulcus from broad posterior lobe; humeral angles rounded, ridge like appearance at the base; lateral margins obliquely straight; posterior margins slightly sinuate; base of pronotum moderately sloping downwards towards scutellum; scutellum triangular, somewhat acute at the apex covered with setae; corium and clavus brownish, covered with fine setae; corium and clavus brownish, covered with fine setae and covered

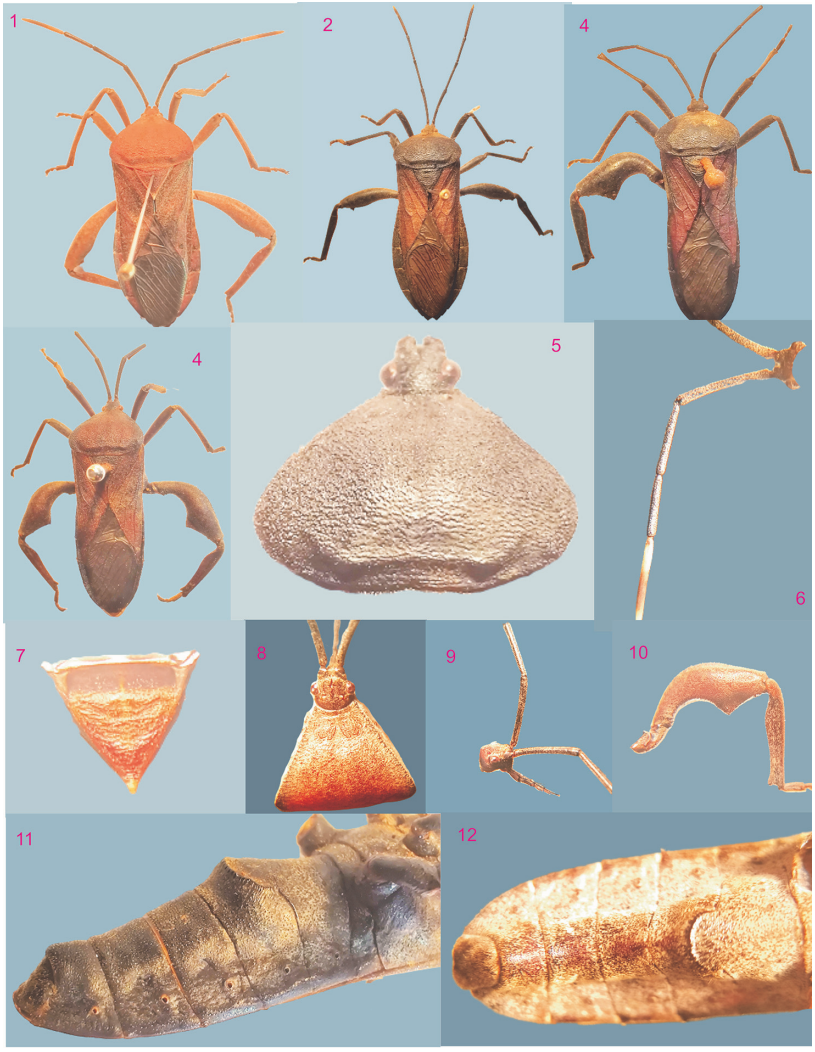


Plate 2: Figures 1-12. Anoplocnemis phasina (Fabricius), 1-2 Dorsal view female (colour variation); 3-4. Dorsal view of male (colour variaTION); 5. Thorax, male; 6. Antennae; 7. Scutellum; 8. Thorax, female; 9. Rostrum; 10. Hind leg, dorsal view; 11-12. Ventral view of abdomen, male (colour variation)

with some apparent punctures; legs black; posterior femora strongly incrassated and curved in the middle with prominent spine and many minute spines; whole dorsal surface granular and setose; the entire surface of tibia slightly granular and setose; posterior tibiae much flattened; membrane brownish, with network like venation, which is not extending beyond the apical segment of abdomen.

**Abdomen:** Abdomen long, elongate and brownish to black; connexivum black and visible; sternum black covered with setae; a semicircular elevated ridge at segments II and III; spiracles large and prominent; the whole area setose, and covered with numerous fine black granules.

In the past, researchers on Coreidae species included Distant (1902), Basu and Mitra (1977 a, b and c, 1978, 1996, 2003, 2004) in India. Coreidae is distributed throughout the world, including countries and Islands such as Archipelago, China, India, Myanmar, Malay Peninsula, Nepal, Sri Lanka, and Thailand (Distant, 1902, 1908 and 1918; Schuh and Slater, 1995; Cassis and Gross, 2002; Dolling, 1991; Aukema *et al.,* 2013). There are over 1,802 species under 252 genera in the world (Schuh and Slater, 1995), out of which 143 species under 45 genera are in India (Distant, 1902, 1908, 1910). *Anoplocnemis phasianus* was noted in Assam, Kerala (Thiruvananthapuram), Madhya Pradesh,

Meghalaya (Khasi hills), Nagaland (Naga Hills), Sikkim, Tamil Nadu (Sholinganallur), Uttarakhand (Gwaldam-Garud Tehsil), and West Bengal (Darjeeling and Buxaduar, Jalpaiguri district). Although *A. phasianus* was recorded from adjacent states such as Maharashtra (Mumbai and Bor Ghat) and Karnataka (Distant, 1902). There is no report of A. phasianus from Goa till date.

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# Efficacy of insecticides against cashew leaf miner, *Acrocercops syngramma* Meyrick (Lepidoptera, Gracillariidae)

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**ABSTRACT:** Experiments were conducted to know the efficacy of different insecticides (Cyantraniliprole 10.26 % OD @ 1.8 ml $L^{-1}$, Dinotefuran 20 % SG @ 0.3 g $L^{-1}$, Chlorfenapyr 10 % SC @ 1.5 ml $L^{-1}$, Imidacloprid 17.8 % SL @ 0.3 ml $L^{-1}$, Thiamethoxam 25 % WG @ 0.3 g $L^{-1}$, Lambda cyhalothrin 2.5 % EC @1 ml $L^{-1}$) against cashew leaf miner, *Acrocercops syngramma* Meyrick under field condition and under nursery condition Among the different insecticides evaluated, under field condition, Lambda cyhalothrin was found to be statistically superior over all other treatments recording 83.39 per cent reduction over control. Imidacloprid was found least effective against leaf miner (46.41% reduction of over control). Under nursery condition, Lambda cyhalothrin recorded 79.13 per cent reduction whereas Imidacloprid recorded only 20.31 per cent. © 2023 Association for Advancement of Entomology

**KEY WORDS**: Field, nursery, lambda cyhalothrin, imidacloprid, leaf miner control

Leaf miner, *Acrocercops syngramma* Meyrick (Lepidoptera, Gracillariidae) is one of the important pests of cashew during the post-monsoon period all over the country. The larvae, after hatching from the eggs, start mining the epidermal layer on the upper surface of the tender cashew leaves as well as tender shoots. As a result of feeding, the affected area forms blistered patches of grayish white colour. When the infested leaves mature, the damage manifests as big holes. Young plants are observed to be more prone to attack by this pest and up to 8 and 15 caterpillars have been observed on a single leaf. During the developmental period, leaf miner larvae are dull white and turn pinkish before pupation. After full development, the larvae fall off to the soil where they pupate and emerge after 7-9 days. The adult is a silvery grey moth that lays eggs on tender leaves (Athalye and Patil, 1999). Leaf miner is a defoliating pest of cashew, occurring in almost all the cashew-growing regions of the country as well as the world. In India, it causes serious damage to the tender leaves of cashew

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attacking 2-80 per cent of the young leaves (Ayyanna *et al*., 1985). Upon hatching, the larva makes a silvery sinuous gallery on the upper leaf side feeding below the epidermal layer, causing leaf blisters which later dry up, causing leaf distortion, browning and curling of the leaves. As many as 11 larvae have been observed feeding on a single leaf (Jena *et al*., 1985). The pest completes its life cycle in a short period of 20-22 days and spreads fast causing leaf blisters over a wide area (Rai, 1984). Over the decades, use of chemical insecticides has become the only line of defense to combat the major pests of cashew (Kar, 2017). Lambda cyhalothrin is a proven insecticide against tea mosquito bug and other associated pests. However, the recent advances in pest management are being directed towards the development of safer and effective insecticides which reduce the pesticide load in the environment.

**Efficacy of insecticides under field conditions:** The experiment was conducted in field conditions at Zonal Agricultural and Horticultural Research Station (ZAHRS), Shivamogga, Karnataka in randomized complete block design (RCBD) with six insecticidal treatments (Cyantraniliprole 10.26 % OD @ 1.8ml $L^{-1}$, Dinotefuran 20 % SG @ 0.3 g $L^{-1}$, Chlorfenapyr 10 % SC @ 1.5ml $L^{-1}$, Imidacloprid 17.8 % SL @ 0.3ml $L^{-1}$, Thiamethoxam 25 % WG @ 0.3g $L^{-1}$, Lambda cyhalothrin 2.5 % EC @1ml $L^{-1}$) and a control, with three replications (one tree is one replication to evaluate insecticides for the management of cashew leaf miner. In each tree, 20 young leaves were randomly selected representing four directions, and in each directions five leaves three from from top and two from middle canopy were selected. Number of leaf miner larvae per leaf were counted and averaged. Pre-treatment count a day before spraying and post treatment counts on three, five, seven and ten days after spraying were recorded. Two sprays were given during the peak incidence in December month. The data were subjected to statistical analysis.

**Efficacy of insecticides at Nursery:** The experiment was conducted in nursery of one and half year age plants at Agricultural and Horticultural Research Station (AHRS), Bavikere, Chikkamagaluru, Karnataka with RCBD with seven treatments including control (as above) with three replications to evaluate insecticides for the management of cashew leaf miner. In each replication, there were ten plants. One spray was given during the peak incidence during November month. Five plants were selected randomly from each replication in each treatment. In each plant, two top leaves were selected randomly. Number of leaf miner larvae were counted and averaged. Pre-treatment count a day before spraying and post treatment counts on three, five, seven and ten days after spraying were recorded.

The statistical analysis of the data obtained from insecticidal efficacy trial was done using analysis of variance (ANOVA) using Web Agri Stat Package (WASP-2) developed by Indian Council of Agricultural Research, Research Complex, Goa. The numerical data were subjected to a square root transformation before subjecting to ANOVA. After analysis, data were fitted in the table as per the needs of objectives for interpretation of results and the data were correlated with the weather parameters following the methods of Gomez and Gomez (1984). The interpretation of data was done by using the critical difference value calculated at 0.05 probability level. Further, the per cent reduction of cashew leaf miner over control was calculated by following formula:

$$\text{Per cent reduction} = \frac{C_b - T_a}{C_b} \times 100$$

Where, $C_b$ = number of insects in untreated control before insecticide application

Ta = number of insects in treated plot after insecticide application

Results of field trial revealed that the incidence of leaf miner (larvae per 20 leaves) ranged from 1.90 to 11.44 in all the treatments including control. The least larval population (1.90 larvae) was recorded in Lambda cyhalothrin treatment, followed by Cyantraliprole (3.48 larvae), Dinotefuran 20 (4.01 larvae), Thiamethoxam (4.65 larvae) and Chlorfenapyr (5.00 larvae). Among the insecticides treated trees, highest mean larval population was

Table 1. Efficacy of insecticides under field condition against Leaf miner, *A. syngramma* on cashew at ZAHRS, Shivamogga during 2021 - 22

| No. | Treatments | Dose per lt | Mean number of leaf miner larvae/20 leaves | | | | | | | | | | Reduction (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | First Spray | | | | | Second Spray | | | | | |
| | | | 1 | 3 | 5 | 7 | 10 | 3 | 5 | 7 | 10 | Mean | |
| 1 | Cyantraliprole 10.26 OD | 1.8ml | 12.80 (3.64) | 6.89 (2.71)$^{c}$ | 5.91 (2.52)$^{cd}$ | 4.55 (2.22)$^{c}$ | 3.11 (1.88)$^{cd}$ | 3.01 (1.87)$^{d}$ | 1.90 (1.54)$^{c}$ | 1.17 (1.29)$^{de}$ | 1.30 (1.33)$^{c}$ | 3.48 | 69.58 |
| 2 | Dinotefuran 20 SG | 0.3g | 12.53 (3.61) | 6.96 (2.72)$^{c}$ | 6.50 (2.62)$^{bcd}$ | 7.31 (2.77)$^{b}$ | 3.51 (1.97)$^{c}$ | 3.21 (1.92)$^{cd}$ | 2.41 (1.69)$^{c}$ | 1.73 (1.49)$^{cd}$ | 0.45 (0.97)$^{e}$ | 4.01 | 64.94 |
| 3 | Chlorfenapyr 10 SC | 1ml | 13.27 (3.71) | 8.79 (3.05)$^{bc}$ | 8.35 (2.97)$^{bc}$ | 7.48 (2.81)$^{b}$ | 4.45 (2.21)$^{bc}$ | 4.10 (2.14)$^{cd}$ | 3.23 (1.91)$^{bc}$ | 2.47 (1.71)$^{bc}$ | 1.12 (1.27)$^{cd}$ | 5.00 | 56.29 |
| 4 | Imidacloprid 17.8 SL | 0.3ml | 12.80 (3.65) | 9.33 (3.12)$^{b}$ | 9.41 (3.14)$^{ab}$ | 8.53 (2.99)$^{b}$ | 6.32 (2.61)$^{b}$ | 5.91 (2.52)$^{b}$ | 4.16 (2.16)$^{b}$ | 3.56 (1.99)$^{b}$ | 1.80 (1.51)$^{b}$ | 6.13 | 46.41 |
| 5 | Thiamethoxam 25 WG | 0.3g | 12.93 (3.66) | 8.57 (3.01)$^{bc}$ | 7.23 (2.73)$^{bc}$ | 7.22 (2.77)$^{b}$ | 4.63 (2.24)$^{bc}$ | 4.23 (2.71)$^{c}$ | 2.45 (1.71)$^{c}$ | 1.99 (1.58)$^{cd}$ | 0.85 (1.16)$^{d}$ | 4.65 | 59.35 |
| 6 | Lambda cyhalothrin 2.5 EC | 1ml | 12.67 (3.62) | 4.90 (2.30)$^{d}$ | 4.12 (2.14)$^{d}$ | 2.52 (1.71)$^{d}$ | 1.32 (1.35)$^{d}$ | 1.14 (1.23)$^{e}$ | 0.72 (1.10)$^{d}$ | 0.35 (0.92)$^{e}$ | 0.10 (0.77)$^{f}$ | 1.90 | 83.39 |
| 7 | Control | - | 14.00 (3.81) | 13.25 (3.70)$^{a}$ | 12.85 (3.65)$^{a}$ | 12.53 (3.60)$^{a}$ | 10.80 (3.36)$^{a}$ | 10.91 (3.37)$^{a}$ | 10.75 (3.35)$^{a}$ | 10.54 (3.32)$^{a}$ | 9.89 (3.22)$^{a}$ | 11.44 | |
| SEm ± | | | - | 0.122 | 0.179 | 0.143 | 0.188 | 0.096 | 0.126 | 0.128 | 0.054 | | |
| CD at 0.05 | | | NS | 0.366 | 0.539 | 0.431 | 0.566 | 0.290 | 0.380 | 0.385 | 0.162 | | |
| CV (%) | | | 5.918 | 6.979 | 10.727 | 8.983 | 14.245 | 7.499 | 11.116 | 12.330 | 6.213 | | |

Note: DBS - Days Before Spray, DAS - Days After Spray, Figures in parenthesis are $\sqrt{x+0.5}$ transformed values;
Means in the columns followed by the same alphabet do not differ significantly by DMRT (P = 0.05).

recorded in Imidacloprid (6.13) sprayed trees. In untreated control, there were 11.44 larvae per 20 leaves. Leaf miner reduction of over control was maximum in Lambda cyhalothrin (83.39%), followed by Cyantraliprole (69.58%) and Dinotefuran (64.94%), Thiamethoxam (59.35%) and Chlorfenapyr (56.29%). The least was recorded in Imidacloprid (46.41%) among the insecticides (Table 1).

In the nursery experiment, the incidence ranged from 1.88 to 9.01 larvae per 10 leaves. Among the insecticides, the least mean number was recorded in Lambda cyhalothrin treatment (1.88 larvae per 10 leaves), followed by Cyantraliprole (3.11), Dinotefuran (4.55), Thiamethoxam (4.77) and Chlorfenapyr (5.39). The maximum mean number was recorded in Imidacloprid (7.18). In untreated control it was 9.01. The reduction leaf miner larvae over the control, was highest in Lambda cyhalothrin (79.13%), followed by Cyantraliprole (65.48%), Dinotefuran (49.50 %), Thiamethoxam (47.05%) and Chlorfenapyr (40.17%). The least was recorded in Imidacloprid (20.31%) among the insecticides (Table 2). Overall, among the insecticides evaluated, Lambda cyhalothrin 2.5 EC @ 1 ml $L^{-1}$ was found most effective in managing the leaf miner, *A. syngramma* in cashew with respect mean number of larvae and percent reduction over the control both in field and nursery conditions.

These results are in line with Kar (2017) and Patel *et al*. (2018) who reported that out of different insecticides evaluated against leaf miner of cashew, Lambda cyhalothrin 5 EC (@ 0.003%) was most efficient which recorded minimum leaf damage in

Table 2. Efficacy of insecticides against Leaf miner, *Acrocercops syngramma* in cashew nursery at AHRS, Bavikere during 2021

| No. | Treatments | Dosage per L | Mean no. of leaf miner larvae/10 leaves | | | | | | Reduction over control % |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1DBS | 3DAS | 5DAS | 7DAS | 10DAS | Mean | |
| 1 | Cyantraliprole 10.26 OD | 1.8ml | 6.99 (2.68) | 5.34 (2.38)$^{bc}$ | 3.21 (1.91)$^{de}$ | 2.61 (1.75)$^{cd}$ | 1.28 (1.33)$^{d}$ | 3.11 | 65.48 |
| 2 | Dinotefuran 20 SG | 0.3g | 9.12 (3.10) | 8.59 (3.01)$^{a}$ | 3.88 (2.07)$^{cde}$ | 3.22 (1.92)$^{c}$ | 2.52 (1.74)$^{c}$ | 4.55 | 49.50 |
| 3 | Chlorfenapyr 10 SC | 1ml | 8.09 (2.92) | 7.59 (2.83)$^{ab}$ | 5.93 (2.53)$^{bc}$ | 4.16 (2.16)$^{bc}$ | 3.88 (2.06)$^{bc}$ | 5.39 | 40.17 |
| 4 | Imidacloprid 17.8 SL | 0.3ml | 7.59 (2.83) | 8.93 (3.06)$^{a}$ | 8.59 (3.01)$^{ab}$ | 6.29 (2.56)$^{ab}$ | 4.90 (2.32)$^{b}$ | 7.18 | 20.31 |
| 5 | Thiamethoxam 25 WG | 0.3g | 7.64 (2.85) | 6.49( 2.58)$^{abc}$ | 5.32 (2.40)$^{cd}$ | 4.23 (2.17)$^{bc}$ | 3.05 (1.87)$^{c}$ | 4.77 | 47.05 |
| 6 | Lambda cyhalot hrin 2.5 EC | 1ml | 6.93 (2.72) | 4.22 (2.16)$^{c}$ | 1.87 (1.53)$^{e}$ | 1.10 (1.26)$^{d}$ | 0.33 (0.91)$^{e}$ | 1.88 | 79.13 |
| 7 | Control | - | 8.01 (2.90) | 9.18 (3.10)$^{a}$ | 9.22 (3.12)$^{a}$ | 9.08 (3.09)$^{a}$ | 8.55 (3.00)$^{a}$ | 9.01 | |
| | | SEm ± | - | 0.193 | 0.185 | 0.179 | 0.128 | | |
| | | CD at 0.05 | NS | 0.580 | 0.556 | 0.538 | 0.386 | | |
| | | CV (%) | 11.68 | 11.928 | 13.216 | 14.198 | 11.486 | | |

Note: DBS - Days Before Spray; DAS - Days After Spray; Figures in parenthesis are $\sqrt{x+0.5}$ transformed values;
Means in the columns followed by the same alphabet do not differ significantly by DMRT (P = 0.05).

the field. In nursery also, the experiment results are in line with Kar (2017) and Patel *et al*. (2018) who reported that Lambda cyhalothrin 5 EC (@ 0.003%) was effective in minimizing the leaf damage. Overall, among the insecticides evaluated both in field and nursery conditions, Lambda cyhalothrin 2.5 EC @ 1ml $L^{-1}$ was found most effective in managing the leaf miner, *A. syngramma* in cashew with respect mean number of larvae and percent reduction over the control.

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# Biology of shoot and fruit borer, *Earias vittella* Fabricius (Noctuidae, Lepidoptera) on okra

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**ABSTRACT:** The biology of fruit and shoot borer (*Earias vittella* F.) was studied under the natural conditions in the instructional farm of Uttar Banga Krishi Viswavidyalay in *pre-kharif*, *kharif* and *post-kharif* on okra. The study revealed that the egg incubation in *pre-kharif, kharif and post-kharif* revealed that the egg incubation were 3.18±0.40, 3.61±0.70 and 4.25±0.67 and larval period were 11.00±0.88, 11.00±0.82 and 13.01±1.81 during corresponding seasons respectively. The pupal period in *pre-kharif, kharif and post-kharif* were 13.01±1.81; 11.00±0.82; 10.97±2.15 days respectively. The adult male longevities observed were 3.64±0.67, 5.60±0.70 and 7.33±0.97 during *pre-kharif, kharif* and *post-kharif.* On the other hand female longevities observed were about 7.45±1.57; 8.87±1.10; 9.41±1.51 days in corresponding seasons respectively. The female fecundity during *pre-kharif, kharif* and *post-kharif* were 91.55±10.93, 117.55b±10.60 and 132.04±5.83 eggs in their lifetime. The pre-oviposition during *pre-kharif, kharif* and *post-kharif* were 1.64±0.50, 1.52±0.53 and 1.88±0.57 and the oviposition periods in corresponding seasons were 2.73±0.47, 3.22±0.79 and 4.32±0.52. While post-oviposition periods in *pre-kharif, kharif* and *post-kharif* were 3.09±0.70, 4.14±0.63 and 3.23±0.92 respectively. Its total life-cycle was completed in 27.45±1.57, 31.60±1.90 and 41.35±2.55 days during *pre-kharif, kharif* and *post-kharif* respectively. Higher fecundity may lead to faster population growth that may surpass the economic threshold level (ETL), even though the shoot and fruit borer's life cycle is longer in *kharif* and *post-kharif*. Thus, it may be said that the *kharif* and *pos-kharif* seasons are the most susceptible to shoot fruit and shoot borer infestation.


**KEY WORDS :** Life cycle, seasons, susceptability, population growth

Okra, *Abelmoschus esculentus* (L.) is a native of tropical or sub-tropical Africa and belongs to the Family Malvaceae. In India, okra is cultivated around the year in *pre-kharif*, *kharif* and *post-kharif* seasons. Okra is attacked by several species of insect pests and infected by a few diseases from seedling to harvesting. Economic losses depend on the degree of damage, pest density, environmental condition, stage of growth and the plant part damaged by the pest. Studying the life cycle of insect pests is vital for effective pest management, sustainable agriculture, environmental protection, and reducing the economic impact of pest damage. It serves as the cornerstone for making well-informed decisions about pest control tactics and aids in balancing the need for pest control with

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ecological and economic considerations. Among different insect pests infesting okra in the terai region of West Bengal, fruit borer and jassid are considered key pests since they are causing regular menace to okra in huge amounts. Therefore, an attempt has been made to study the biology of the fruit and shoot borer *(Earias vittella F.)* as influenced by the seasons.

A laboratory culture of *E. vittella* was started with several infested okra pods collected from the field which were transferred into plastic jars covered with muslin cloth and kept in laboratory conditions till pupation and adult emergence. After mating adults were shifted to potted okra plants with young fruit and covered with net for egg laying. The egg masses were examined daily and the incubation period was recorded. On hatching, the larva was monitored until the end of the last instar. The full-grown larva bores a large exit hole, leaves the okra pod, spins a cocoon and pupates on the outer surface of the fruit. The duration of the larval and pupal stages was determined. For studying adult longevity, 10 pairs of newly emerged males and females were maintained in glass vials with 10% sugar solution in absorbent cotton. The time elapsed between the emergence of each moth and its death was recorded as the longevity of such an individual. The duration of each generation was estimated based on the average length of the life cycle. The duration of pre-oviposition, oviposition, and post-oviposition were recorded for the female. The cage was opened daily and the leaves and fruits were observed for oviposition with the help of magnifying lens and this was continued till the last egg was laid. SAS software (ver. 9.2) was used for data analysis. One-way ANOVA was performed for each of the parameters and separation of the means was done using the Least Significant Difference (LSD) test at 5% significant level.

**A. Incubation period**

The incubation period varied in different crop seasons. The highest incubation period of 4.25±0.67 days was recorded in *post-kharif* followed by 3.61±0.67 days in *kharif* and the shortest duration (3.18±0.40 days) was recorded in *pre-kharif.* This result is in agreement with Rehman and Ali (1981) in January to March; Singh and Bichoo (1989) in September to October; Raju (2016) in *kharif* and Nishi (2016) in March-April who reported that the incubation period lasted for 3 to 4 days. The incubation period was 3.27 and 3.67 days as reported by Kumar *et al.* (2014) during July-August and October-November supports our study. At par with the present result for the *post-kharif* season, Das and Chaudhuri (2012) recorded 4.50 days of incubation period in October-November. A similar observation of 4.57 days and 5.00 days was also observed by Sundraraj and David (1987) and Suryawanshi *et al.* (2001). Bhat *et al*. (2005) recorded 2.60-3.80 days during June-August and 4.40-4.80 days during September-November which supports the present observation. The results under present studies as well as other reports as discussed above contradict Al-Mehmmady (2000) who found shorter incubation of 2.15 and 2.42 days in August and September. In line with Al-Mehmmady (2000) and Syed *et al*. (2011) also found 2.30 days of incubation period.

**B. Larval period**

The larva is the most injurious stage and therefore, their growth and development is very important, as it directly affects fruit production. Caterpillar passed through five stages. The duration of different larval instars varied with seasons. Total larval periods of 11.00±0.88; 12.25±1.20 and 17.90±1.17 days were recorded in *pre-kharif, kharif* and *post-kharif* respectively. In conformity with the present study during *post-kharif* Rukhsana *et al*. (1995), Suryawanshi *et al.* (2001) and Das and Chaudhuri (2012) also recorded larval periods of 18.00±0.88, 19.00 and 16.66-23.33 days respectively. Other workers reported similar result where the period lasted for 9.30 days (Ambegankar and Billapate, 1984); 12.73 days (Sundraraj and David, 1987); 11.28-11.39 days (Al-Mehmmady, 2000); 10-12 days (David, 2002); 7-15 days (Srivastava, 2003); 9.20-11.20 days (Dhillon and Sharma 2004); 8.20-9.00 days in June-August and 9.80-12.20 days in September to November (Bhat *et al*., 2005); 9.16 days (Syed *et al*., 2011); 8.00 days in March-August (Shah *et al*., 2012); 11.79 days in July-

Table 1: Duration of different developmental stages of *Earias vitella* on okra over seasons

| | *Pre-kharif* | *Kharif* | *Post-kharif* | | | | |
|---|---|---|---|---|---|---|---|
| Mean temperature (Min-max) | 25.08°C (20.60-31.55 °C) | 28.08 °C (23.65-32.50 °C) | 27.37 °C (21.92-32.82 °C) | | | | |
| Mean RH (Min-Max) | 74.28% (72.97-79.71 %) | 84.26% (78.69-87.83 %) | 78.45% (74.73-82.16%) | | | | |
| Life stages | Duration in days (Mean±SD) | | | | | | |
| | *Pre-Kharif* | *Kharif* | *Post-Kharif* | Average | F | Pr>F | LSD |
| Incubationperiod | 3.18±0.40b | 3.61±0.70b | 4.25±0.67a | 3.68±0.57 | 8.58 | 0.0020 | 0.539 |
| Larval period | | | | | | | |
| 1st instar | 1.09±0.30c | 1.60±0.52b | 2.15±0.42a | 1.61±0.56 | 17.69 | <.0001 | 0.370 |
| 2nd instar | 2.00±0.09b | 2.03±0.20b | 3.04±0.57a | 2.36±0.64 | 30.07 | <.0001 | 0.318 |
| 3rd instar | 2.18±0.40b | 2.34±0.48b | 3.61±0.67 a | 2.71±0.85 | 21.84 | <.0001 | 0.494 |
| 4th instar | 2.36±0.50c | 2.85±0.42b | 4.76±0.32a | 3.32±1.36 | 68.11 | <.0001 | 0.454 |
| 5th instar | 3.36±0.50b | 3.43±0.70b | 4.35±0.52a | 3.71±0.59 | 17.45 | <.0001 | 0.387 |
| Total Larva | 11.00±0.88b | 12.25±1.20b | 17.90±1.17a | 13.80±3.94 | 51.74 | <.0001 | 1.508 |
| Pupal Period | 8.91±0.54c | 11.00±0.82b | 13.01±1.81a | 10.97±2.15 | 32.63 | <.0001 | 1.059 |
| Adult longevity | | | | | | | |
| Male | 3.64±0.67c | 5.60±0.70b | 7.33±0.97a | 5.52±1.91 | 58.27 | <.0001 | 0.714 |
| Female | 7.45±1.57b | 8.87±1.10a | 9.41±1.51a | 8.58±2.05 | 7.10 | 0.0047 | 1.231 |
| Life cycle | 27.45±1.57a | 31.60±1.90b | 41.35±2.55a | 33.46±1.70 | 67.10 | <.0001 | 2.568 |
| Fecundity | 91.55±10.93c | 117.55b±10.60b | 132.04±5.83a | 113.72±21.33 | 54.71 | <.0001 | 8.183 |
| Pre-oviposition | 1.64±0.50a | 1.52±0.53a | 1.88±0.57a | 1.68±0.42 | 1.42 | 0.2648 | 0.458 |
| Oviposition | 2.73±0.47 | 3.22±0.79 | 4.32±0.52 | 3.42±0.56 | 20.47 | <.0001 | 0.528 |
| Post-oviposition | 3.09±0.70b | 4.14±0.63a | 3.23±0.92a | 3.48±0.19 | 5.51 | 0.0124 | 0.717 |

** Within row means followed by the same letter(s) are not significantly different at 5% level*

August and 11.15 days in October-November (Kumar *et al*., 2014) and 11.50±1.08 days in March-April (Nishi, 2016). The newly hatched larvae wandered about for few hours before boring into the fruit. The period of different larval instars were 1.09±0.30, 2.00±0.09, 2.18±0.40, 2.36±0.50 and 3.36±0.50 days in *pre-kharif*; 1.60±0.52, 2.03±0.20, 2.34±0.48, 2.85±0.42 and 3.43±0.70 days in *kharif*; 2.15±0.42, 3.04±0.57, 3.61±0.67, 4.76±0.32 and 4.35±0.52 days in *post-kharif* season respectively. The average duration of different larval stages in the three seasons were 1.61±0.56, 2.36±0.64, 2.71±0.85, 3.32±1.36 and 3.71±0.59 days. This finding was at par with Sewak (2016) where the average duration of 1st, 2nd, 3rd, 4th and 5th larval instars was 1.60±0.52, 2.00±0.00, 2.50±0.53, 2.50±0.53 and 3.00±0.00 days respectively. In support of the present finding during *post-kharif,* Das and Chaudhuri (2012) recorded 3.33, 3.00, 3.00, 3.33 and 4.00 days of development period of different larval instars during October-November.

## C. Pupal period

At the end of its development, the larva leaves the fruits and settles down to spin their cocoons at different places outside the okra fruits. Pupation occurred in a dirty white boat-shaped cocoon. The average pupal period was 10.97±2.15 days in three

seasons. The longest period was observed in *post-kharif* (13.01±1.81 days) followed by *kharif* (11.00±0.82 days) and the shortest (8.91±0.54 days) was in *pre-kharif*. In support of the present findings other workers recorded a period of 6-14 days (Rehman and Ali, 1981 and Singh and Bichoo, 1989); 11.16 days (Sundraraj and David, 1987); 6.45 and 7.78 days during August-October (Al-Mehmmady, 2000); 10.00 days (Suryawanshi *et al.,* 2001); 7-10 days (David, 2002); 7.8-8.6 days during June-August and 9.8-10.2 days during September-November (Bhat *et al.*, 2005); 10.00 in July and 11.80 days in September (Syed *et al.*, 2011) and 8.0±0.82 days (Nishi, 2016). The pupal period lasted for 8.50-9.50 days in October-November as reported by Das and Chaudhuri (2012) deviates from the present work, particularly for *post-kharif* crop.

**D. Adult stage**

The duration of different stages of *Earias* adult life is presented (Table 1). The adult longevity was highest in *post-kharif* (7.33±0.97 days for males and 9.41±1.51 days for females) and it was supported by the findings of Suryawanshi *et al.* (2001) where it was 10 days and Das and Chaudhuri (2012) with 9.16 days of adult longevity. The male and female adults during *kharif* lived for 5.60±0.70 and 8.87±1.10 days and it was 3.64±0.67 and 7.45±1.57 days in *pre-kharif* confirms the report of Shah *et al*. (2012) and (Nishi, 2016) who recorded the period as 6-12 days and 9-14 days and 4.2 days and 9.5 days for male and female respectively. The average life span of male and female moths in three generations was 5.52±1.91 days and 8.52±2.05 days respectively. However, longer male and female adult longevity was recorded by different workers and the duration was 9.25 and 13.91 days (Rehman and Ali, 1981); 10.76 and 14.60 days (Sundraraj and David, 1987); 12.45 and 14.00 days during August and 13.36 and 14.20 days during October (Al-Mehmmady, 2000) and 13.9 and 14.2 days in July, 11.66 and 13.3 days in September and 8.0 and 11.8 days in October (Syed *et al*., 2011). This might be due to variations in regional climatic conditions influencing the activity of the insect.

The pre-oviposition, oviposition and post-oviposition period were 1.64±0.50, 2.73±0.47 and 3.09±0.70 days in *pre-kharif*; 1.52±0.53, 3.22±0.79 and 4.14±0.63 days in *kharif* and 1.88±0.57, 4.32±0.52 and 3.23±0.92 days in *post-kharif* with an average of 1.68±0.42, 3.42±0.56 and 3.48±0.19 days respectively. Das and Chaudhuri (2012) also recorded the 1.00-2.00 days of pre-oviposition, 5.00-5.15 days of oviposition and 2.00-2.05 days of post-oviposition period in October-November. The earlier work by Bhat *et al*. (2005) revealed that the pre-oviposition period varied from 1.8 to 2.44 days in August to 3.6 ± 0.54 days in November, whereas the oviposition and post-oviposition period ranged from 2.2±0.44 (August) to 4.2 ± 0.44 in November and 8.4 ± 0.54 in June to 12.2 ± 0.83 in November. However, Suryawanshi *et al.* (2001) recorded a longer oviposition period of 7 days and the post-oviposition period was only 1.00 days. In support of *the pre-kharif* result, Nishi (2016) also obtained 1.8±0.78, 2.8±0.79 and 4.5±0.53 days of pre-oviposition, oviposition and post-oviposition period during March-April while Rehman and Ali (1981) reported 3.5, 5.83 and 4.75 days respectively. The corresponding values were 1.5, 7.0 and 10.0 in July, 0.66, 5.0 and 3.0 in September and 1.75, 4.5 and 2.0 in October respectively as recorded by Syed *et al*. (2011). However, Shah *et al*. (2012) reported 6-8 days of egg laying period.

**E. Life cycle**

The duration of the life cycle varied with the seasons. In *post-kharif*, the duration was longest (41.35±2.55 days) followed by kharif (31.60±1.90 days) and shortest (27.45±1.57 days) in *pre-kharif*. The present findings are in close conformity to Al-Mehmmady (2000) who reported life cycle of 39.00 to 49.40 days, 31.82 to 36.59 days and 35.40-39.64 days during February-May, June-August and September to December. Sharma *et al.* (1985) recorded the duration ranged from 29 to 49 days which supports the present result. The period of 32.50±3.24 days as reported by Nishi (2016) during March-April also confirms the present result. Rehman and Ali (1981) reported a total life span of 24-45 days at 36.7°C. However, a shorter period was recorded by Nayar *et al.* (1976) (20-22 days);

Butani and Jotwani (1984) (22 to 25 days) and Sundraraj and David (1987) (26.9 days). Sharma *et al.* (1985) found that dry and cold weather prolongs the duration of the different stages resulting in prolonged duration of generation, whereas humid and warm weather is considered favorable for the growth and development of this insect as it completes its generation in a shorter time. It could be concluded that temperature influences the development of the moth which is inversely proportional to it (Table 1).

**F. Fecundity**

The longevity of adult female is an important factor in the realization of its oviposition potential. The fecundity was highest in *post-kharif* 132.45±5.83 eggs/female followed by *kharif* (117.55±10.60 eggs/female) and *pre-kharif* (91.55±10.93 eggs). In support of the present result Das and Chaudhuri (2012) reported that the adult female of the October-November generation laid 135 eggs. However, a higher fecundity of 200-400 eggs/female was recorded by Atwal and Dhaliwal (2005); Panwar (2002) and Srivastava (2003). David (2002) also recorded higher fecundity of 385 ggs/female. According to Bhat *et al.* (2005) highest fecundity was recorded during September (206.40 eggs/female) and the lowest in June (187.4 eggs/female). In support of Bhat *et al.* (2005), Kumar *et al.* (2014) also revealed that the fecundity was 196 eggs during July-August as compared to 203 eggs during October-November. Nishi (2016) found that the fecundity was 199±34.3eggs in March-April. Each female laid about 277 eggs and 150-250 eggs singly as reported by Syed *et al.* (2011) and Shah *et al.* (2012). Although, the fecundity of fruit borer in aforesaid works is not in conformity with the present investigation trends in seasonal variation are at par with the results present study.

Shoot and Fruit borer life cycles were shorter during *pre-kharif* (27.45 days), indicating that there were more numbers of generations, but because of the low fecundity (91.00 eggs/female), population expansion may have been slower. The Fruit and Shoot Borer took 31.60 days in *kharif,* which is longer than in *pre-kharif*, but its higher fecundity (117.55 eggs/female) may lead to a large population expansion. The longest life cycle was recorded in *post-kharif* season (44.10 days) but higher fecundity (133.00 days), may lead to higher population growth than in the *pre-kharif* and *kharif* seasons. It can be concluded *post-kharif* seasons is most vulnerable to okra fruit and shoot bore followed by *kharif* and *pre-karif*.

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# *Lysinibacillus fusiformis:* A novel mosquitocidal bacterium isolated from Western Ghats, Kerala, India

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**ABSTRACT:** An extensive field survey was carried out from December 2021 to January 2022 for the collection of soil samples in the Western Ghat region of the Wayanad district of Kerala, India, to isolate potent novel mosquitocidal bacteria which could be used as efficient formulations to control mosquito vectors. Several bacterial colonies were isolated and screened, for mosquitocidal activity. Toxicity assay showed that only one bacterium isolated from forest loamy soil had promising larvicidal activity against *Culex quinquefasciatus* the lymphatic filariasis vector ($LC_{50}$: 0.03mg $L^{-1}$ and $LC_{90}$: 0.07mg $L^{-1}$) and moderate activity was shown against the dengue vector, *Aedes aegypti* ($LC_{50}$:1.03 mg $L^{-1}$ and $LC_{90}$: 1.8 mg $L^{-1}$). The bacterium was identified as *Lysnibacillus fusiformis* by a phylogenetic tree constructed using 16S rRNA genome sequencing. This is the first report that *L. fusiformis* isolated from the forest loamy soil of the Western Ghats, Kerala, which showed proficient mosquitocidal activity against disease-transmitting mosquito vectors. 

**KEYWORDS:** *Culex quinquefasciatus*, *Aedes aegypti,* soil, genome sequencing, first report, toxicity assays

Mosquito-borne diseases (MBD), such as dengue, malaria, and Zika virus fever, chikungunya, pose severe risks to the public's health (WHO, 2020). Vector control is crucial for reducing the epidemics of several MBDs, limiting disease transmission, and improving the quality of life. The resurgence of vector-borne diseases as a result of favorable environmental factors, such as rapid urbanization, the development of vector resistance to insecticides, and human lifestyle changes encourage the need for the adoption of safer and more efficient vector control techniques (Chediak *et al.,* 2016; Garcia *et al.,* 2018). The WHO promotes integrated vector management (IVM), which emphasizes the use of long-lasting, eco-friendly alternative vector control measures. Biological control measures, including bacterial pesticides, are found to be a better alternative since they are eco-friendly, cost-effective, and target-specific. To control mosquito vectors, *Bacillus thuringiensis israelensis (Bti)* and *B. sphaericus (Bs)* bacteria are frequently used (Prummongkol *et al*., 2019; Vimala Devi *et al*., 2021). However, the long-term usage of these bio-control agents like *Bs* resulted in the occurrence

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of resistance in vector mosquitoes (Guidi *et al*., 2013; Su *et al*., 2019). This challenging situation prompted the researchers to look for novel mosquitocidal bacteria from natural sources. The Western Ghats in Kerala are renowned for their diverse and unique collection of fauna and flora. Soil is the richest source of microbes and there have been numerous reports of mosquitocidal bacteria isolated from soil in different parts of the world (Nair *et al*., 2020; Iftikhar *et al*., 2023). Whereas the soil of the Western Ghat region of Kerala has not been explored for isolation of mosquitocidal bacteria.Therefore, the present study aimed to isolate novel mosquitocidal bacteria from various soil types collected from the Western Ghats, Wayanad, Kerala, India.

The soil samples were collected from tea plantations, coffee plantations, and forests in the Wayanad district, which includes parts of the Western Ghats (11.68°N; 76.13°E). The soil sampling was done from December 2021 to January 2022. In each sampling spot, the surface layer, 5 and 10 centimeters below the soil, were collected and pooled together in sterile vials. Soil types were documented. Samples were brought into the laboratory and processed by serial dilution and spread plate method. A required amount of soil sample was weighed (1g) and serially diluted ($10^{-3}$) in 10 ml of sterile water, and 0.1ml of this serially diluted sample was evenly spread on Nutrient Yeast Salt Mineral (NYSM) agar plates (spread plate method) and these plates were incubated for 24 hours at room temperature. Bacterial colonies were inoculated in 10 ml NYSM broth and incubated in an Orbitek shaker at 250 rpm for 72 hours.

The preliminary toxicity assay (bioassay) was carried out as recommended by WHO guidelines (WHO, 2005) using 72 hours of bacterial culture to screen for mosquitocidal activity. The bioassays were conducted in paper cups (wax-coated) having 100 ml of tap water (chlorine-free) and 25 late third instar larvae of *Culex quinquefasciatus* and *Aedes aegypti* were released into the cups and acclimatized. A dose of 1µl of 72 hours of bacterial culture was used to treat the larvae in the cups, while controls without the addition of the bacterial culture were maintained. After 24 hours of exposure, the bioassay results were recorded by scoring the live larvae in respective cups. The bacterial isolate with 100 percent mortality of larvae was considered potential bacteria, and stored in a deep freezer as glycerol stock (30%) until further use. The potential mosquitocidal bacterium was subjected to extensive bioassay /detailed toxicity assay to determine the lethal concentration values ($LC_{50}$ and $LC_{90}$). Bacterial glycerol stocks were inoculated in 10 ml of NYSM broth, kept for incubation overnight at 250 rpm in an Orbitek shaker, and sub-cultured in 100 ml of NYSM broth for 72 hours. After complete sporulation, the bacterial cell pellets were separated by centrifugation (10000g, 20 min) (Hitachi, Japan) and then lyophilized (Christ ALPHA 1-2 LD plus, Germany). A homogenized bacterial stock solution was prepared (5mg/10 ml) using lyophilized powder. Seven different doses of this stock solution were used for conducting the extensive bioassay. Four replicates for each dose were maintained in every experiment with appropriate controls (WHO, 2005). After 24 hours, the result was scored by counting the live larvae. The experiment was repeated three times to ensure reproducibility. The results were finally analyzed by Probit analysis using SPSS.16.0 software.

Table 1. Mosquitocidal activity of *L. fusiformis*

| Mosquito species | $LC_{50}$ (mg $l^{-1}$) | $LC_{90}$ (mg $l^{-1}$) (LCL*-UCL*) | Slope (LCL*-UCL*) | Intercept | $X^2$ |
|---|---|---|---|---|---|
| *Culex quinquefasciatus* | 0.03 (0.025-0.039) | 0.07 (0.068-0.081) | 0.006 | -1.160 | 89.879 |
| *Aedes aegypti* | 1.02 (0.94-1.1) | 1.8 (1.69-1.9) | 0.002 | -1.714 | 17.864 |

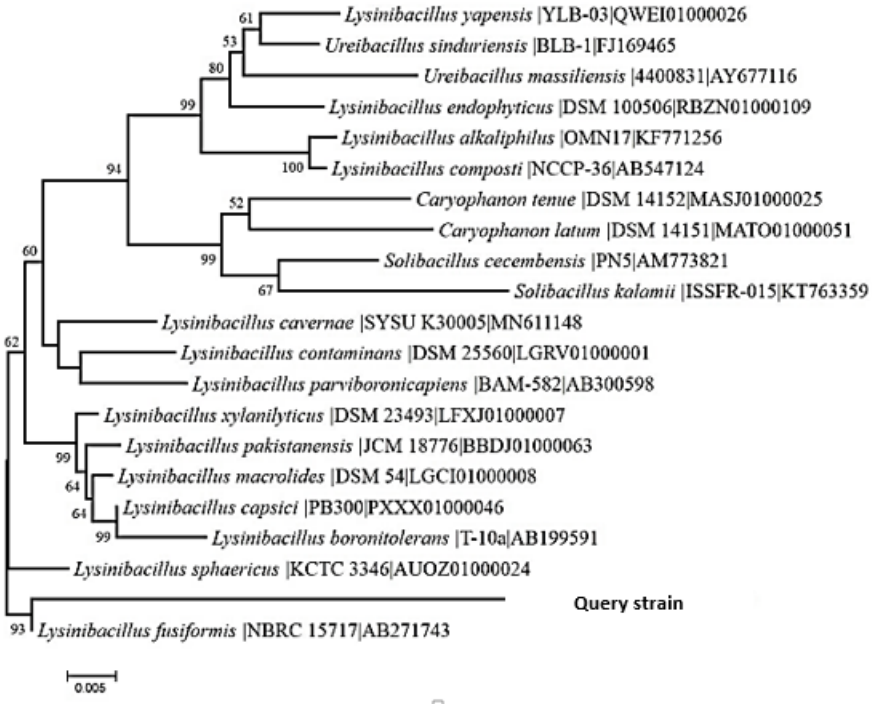


Fig. 1 Phylogenetic tree constructed using 16S rRNA genome of the newly isolated mosquitocidal bacterium (Neighbour joining model)

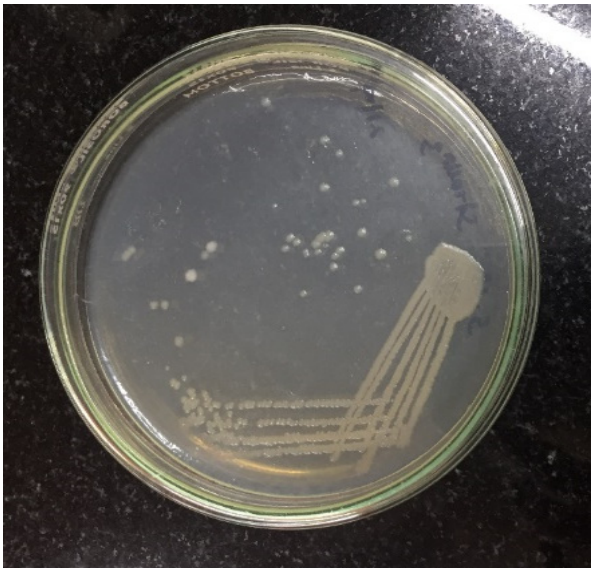

Fig. 2 Colony morphology of *L. fusiformis*

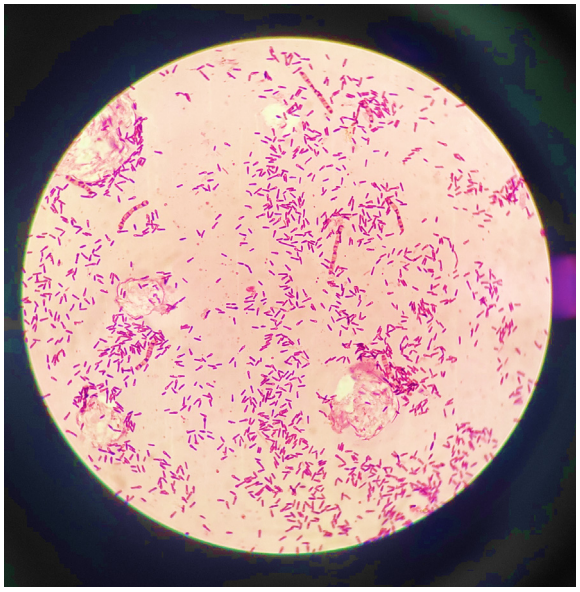

Fig. 3 Vegetative cells of *L. fusiformis* after Gram staining

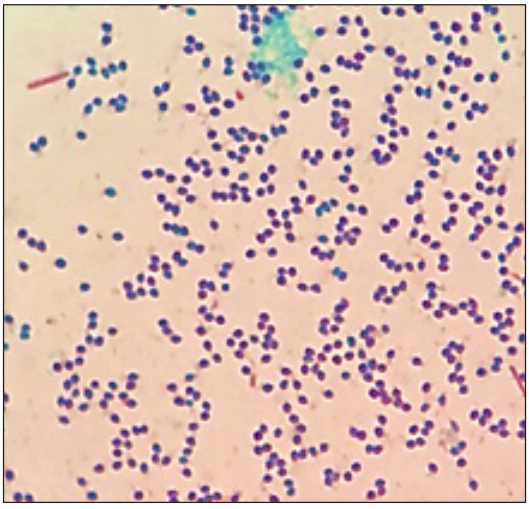

Fig. 4 Spores of *L. fusiformis* after Safranin-Malachite green endo spore staining

The characteristics of bacterial colonies such as the shape, margin, opacity, and color of the bacterial isolate, were observed in the NYSM plate culture. Gram-staining was done to study whether the bacteria were Gram-negative or positive. Spore staining, also known as Schaeffer-Fulton staining, was done to visualize the presence of spores through a microscope (Olympus CX41RF Binocular Microscope, Japan).

For the identification of the mosquitocidal bacterium the genomic DNA was extracted using the GenEluteTM Bacterial Genomic DNA Kit (Sigma Aldrich, USA), and polymerase chain reaction (PCR) was done to amplify the genomic DNA with universal forward and reverse primers (16S rRNA). The Qiaquick PCR purification kit (QIAGEN, USA) was employed to purify the amplified products, which then served as a template for forward and reverse sequencing. The BigDye Version 3.1 kit from Applied Biosystems was utilized to do the DNA sequencing on the ABI-PRISM 3730 DNA sequencer and the contigs sequences were assembled by Bio-Edit (Version 7.0.9.0) and examined via Mascot Server 2.4. BLAST program (NCBI), which was used to identify the species. The 16S rRNA genome sequence was combined with the closely related sequence, and a next-generation phylogenetic tree was constructed using MEGA 5 using the K2P model and 1000 bootstrapping.

The Western Ghats in the states of Kerala were granted a "heritage tag" from UNESCO as they are the "gene pool" that harbors millions of Millions of species. The soil in this region is an excellent source of numerous unique microorganisms that might be investigated for potential applications. There has been limited research on the isolation of mosquitocidal bacteria from the soils of the Western Ghats region of Kerala. (Nampoothiri *et al*., 2013). In the present study, explorative research was carried out by collecting various soil types from the Western Ghats of Wayanadu District of Kerala for isolating novel mosquitocidal bacteria A total of 180 soil samples were collected, and several bacteria were isolated. Among these 12 bacterial strains showed mosquitocidal activity and among these only one bacterium isolated from forest loamy soil had promising larvicidal activity against *Culex quinquefasciatus* the lymphatic filariasis vector ($LC_{50:}$ 0.03mg $L^{-1}$ and $LC_{90}$: 0.07mg $L^{-1}$) and moderate activity was shown against the dengue vector, *Aedes aegypti* ($LC_{50}$: 1.03mg $L^{-1}$ and $LC_{90}$: 1.8mg $L^{-1}$) (Table 2). This potential bacterial strain was identified as *Lysnibacillus fusiformis* by constructing the phylogenetic tree using the 16S rRNA genome sequence (Fig. 1). The colony

morphology of *L. fusiformis* was circular, dry, flat, and rough, with a dull white color (Fig. 2). Microscopic studies on the vegetative stage of the bacterium found it to be rod-shaped and Gram-positive (Fig. 3). The strain was endospore-forming with terminal spherical spores (Fig. 4).

Ramalakshmi and Udayasuriyan (2010) reported different of *Bt* strains from the soil samples of the Western Ghats extension in Tamil Nadu.Similarly, Ganesan and his co-workers (2018) reported *Streptomyces enissocaesilis* (S12-17) from soils of the Western Ghats of Tamil Nadu with effective mosquito larvicidal, ovicidal, and repellent activity (Ganesan *et al.,* 2018).It is the first report of *L. fusiformis* from the soil of Western Ghats, Kerala having mosquitocidal activity. It is a naturally occurring bacterium in the family Bacillaceae and was first discovered in 1901 (Pinheiro *et al.,* 2022). *L. sphaericus* was widely used for mosquito control whereas; the larvicidal activity of *L. fusiformis* has not been reported so far. The underlying mechanism *i.e.,* the toxin (s) responsible for mosquito toxic effect and its mode of action are yet to be studied. In conclusion, this study reports a novel mosquitocidal bacterium, namely, *L. fusiformis* for the first time. It is suggested that the strain may be useful for the control of disease-transmitting mosquito vectors in the current scenario of resistance to some bio-pesticides.

## ACKNOWLEDGMENTS

The first author acknowledges the University Grant Commission (UGC), Ministry of Human Resource and Development, Government of India, for the award of a Senior Research Fellowship (Ref: UGC/191620033726). The authors acknowledge the Director, ICMR-Vector Control Research Centre, Puducherry for proving the facilities and RIU approval. This is part of PhD thesis (Ref: PhD/PU/20201129001, 12/04/2021) for the first author under the guidance of the corresponding author (SP).

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# New faunistic records of two species of pygmy backswimmers (Hemiptera, Heteroptera, Pleidae) from Kerala, India

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**ABSTRACT:** Members of the family Pleidae are commonly referred to as pygmy backswimmers. They are small predatory bugs; usually reside in stagnant or slow moving aquatic habitats with plenty of vegetation. Two species of aquatic insects *Paraplea frontalis* (Fieber, 1844) and *P. liturata* (Fieber, 1844) in the family Pleidae are reported for the first time from Kerala. Both species are relatively common and widespread in India. However, the present faunistic records shall add to the database of geographical distributional range of these species. © 2023 Association for Advancement of Entomology

**KEYWORDS:** Aquatic bugs, *Paraplea frontalis, P. liturata,* nepomorpha, distribution

Pygmy backswimmers or pleids are small globular bugs generally seen in vegetated areas of lentic water bodies (Andersen and Weir, 2004; Chen *et al.,* 2005). As the name indicates, they swim in the inverted or back position. World fauna of the family Pleidae is represented by 37 species under three genera namely, *Plea* Leach, 1815, *Neoplea* Esaki and China 1928 and *Paraplea* Esaki and China 1928. Morphotaxonomic identification of different genera of the family Pleidae is primarily based on the number of tarsal segments. The genus *Paraplea* has two tarsal segments on their fore tarsi and hind tarsi (Cook, 2017; Cook *et al.,* 2020). *Paraplea* is the biggest and extensively distributed genus among pleids (Schuh and Slater, 1995). It is generally worldwide in distribution, but it has not been reported yet from the Palearctic and Antarctic regions (Cook, 2017). Since information on the systematics and detailed bioecology of aquatic bugs from the state of Kerala is sparse, there is scope for future research regarding the taxonomy and bionomics of true bugs in the family Pleidae. Therefore, an endeavour was made to study water bugs from Sasthamkotta lake, the largest freshwater lake of Kerala. Two species of pygmy backswimmers *Paraplea frontalis* (Fieber, 1844) and *P. liturata* (Fieber, 1844) are hereby reported for the first time from Kerala.

The examined specimens were collected from Sasthamkotta lake, Kollam District, Kerala, by using a D-frame aquatic net with a mesh size of 500µm. The collected samples were sorted, and then preserved in 70 per cent ethanol. The adult specimens were taken for taxonomic study. Observations and measurements were done using Olympus SZ51 stereomicroscope. Photographs of the specimens were taken using Olympus TG- 6

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digital camera and later identified with the help of standard literatures and monograph (Bal and Basu, 2000; Nahar, 2004; Thirumalai, 2004; Nieser *et al.,* 2005; Thirumalai and Suresh Kumar, 2005; Jehamalar and Chandra, 2016; Basu *et al.*, 2018; Mitamura *et al.,* 2018; Cook, 2020).

Order Hemiptera Linnnaeus, 1758

Suborder Heteroptera Latreille, 1810

Infraorder Nepomorpha Popov, 1968

Superfamily Pleoidea Fieber, 1851

Family Pleidae Fieber, 1851

Genus ***Paraplea*** Esaki and China, 1928

**1. *Paraplea frontalis* (Fieber, 1844)**

1844. *Ploa frontalis* Fieber, *Entomologische Monographien, Leipzig,* 18.

1898. *Plea frontalis* Fieber: Kirkaldy, *Wien. Ent. Zeit.,* 17: 141.

1906. *Plea frontalis* (Fieber): Distant, *Fauna of British India*, 3: 48.

1934. *Plea (Paraplea) frontalis* (Fieber): Lundblad, *Archiv für Hydrobiologie Supplement*, 12:138.

1947. *Plea (Paraplea) frontalis* (Fieber): Hafiz & Pradhan, *Rec. Indian Mus*., 45: 349

1995. *Paraplea frontalis* (Fieber): Polhemus *et al.*, *Cat. Het. Palaerctic region,* 1: 74.

Materials examined: 2 ♂, 1 ♀, Sasthamkotta Lake; Vettolikadavu, Kollam district, Kerala, 8m amsl., 9°1'56.93" N; 76°37'29.72" E, 18.xii.2022, Coll. K. Jyothylakshmi and S. Nandakumar.

Diagnosis: Body length: 1.8- 2mm; colour: golden brown to dark brown; body prominently punctuate, honeycomb pattern; eyes reddish brown; a small dark reddish-brown longitudinal stripe or light brown bar between the eyes; vertex with two pair of dark blotches or spots, it may be indistinct or absent in some specimens; pronotum wider than long; scutellum with distinct dark punctures; Hemelytra with very fine dark punctures; legs yellowish brown; thoracic keels differently shaped, with spine like projection; male paramere with tuft of setae; female ovipositor, triangular in shape with numerous spines (Figs. 1A- E).

Distribution: India: Andaman and Nicobar Islands, Andhra Pradesh, Arunachal Pradesh, Bihar, Chandigarh, Chhattisgarh, Karnataka, Madhya Pradesh, Maharashtra, Odisha, Pondicherry, Punjab, Tamil Nadu, Uttar Pradesh and West Bengal.

Bionomics: The species is very specific to stagnant or slow moving water bodies with considerable vegetation. They are often seen at the interface between water and vegetation mats, sometimes found associated with the emergent macrophytes. Colour polymorphism can be seen among different individuals of the species. Different colour morphs of *Paraplea frontalis* have been collected. The colour ranges from light brown to dark brown and yellowish brown to golden yellow. This difference in colour might be attributed by the habitat and microhabitat conditions. The species have agile movement and predators that feed primarily on mosquito larvae and other small invertebrates.

Remarks: This is the first record of *Paraplea frontalis* (Fieber, 1844) from Kerala. *Paraplea frontalis* was first named as *Ploa frontalis* in the description made by Fieber (1844). The species is relatively wide spread.

**2. *Paraplea liturata* (Fieber, 1844)**

1844. *Ploa frontalis* Fieber, .*Entomologische Monographien, Leipzig,* 19.

1904. *Plea frontalis* Kirkaldy, *Wien. Ent. Zeit*. 23: 129

1906. *Plea liturata* (Fieber), Distant*, Fauna British India*, 3: 47.

1910. *Plea metiadusa* Distant, *Fauna Brilish India*, 5: 337.

1934. *Paraplea liturata* (Fieber): Lunblad. *Arch. Hydrobiol. Suppl*., 12: 129.

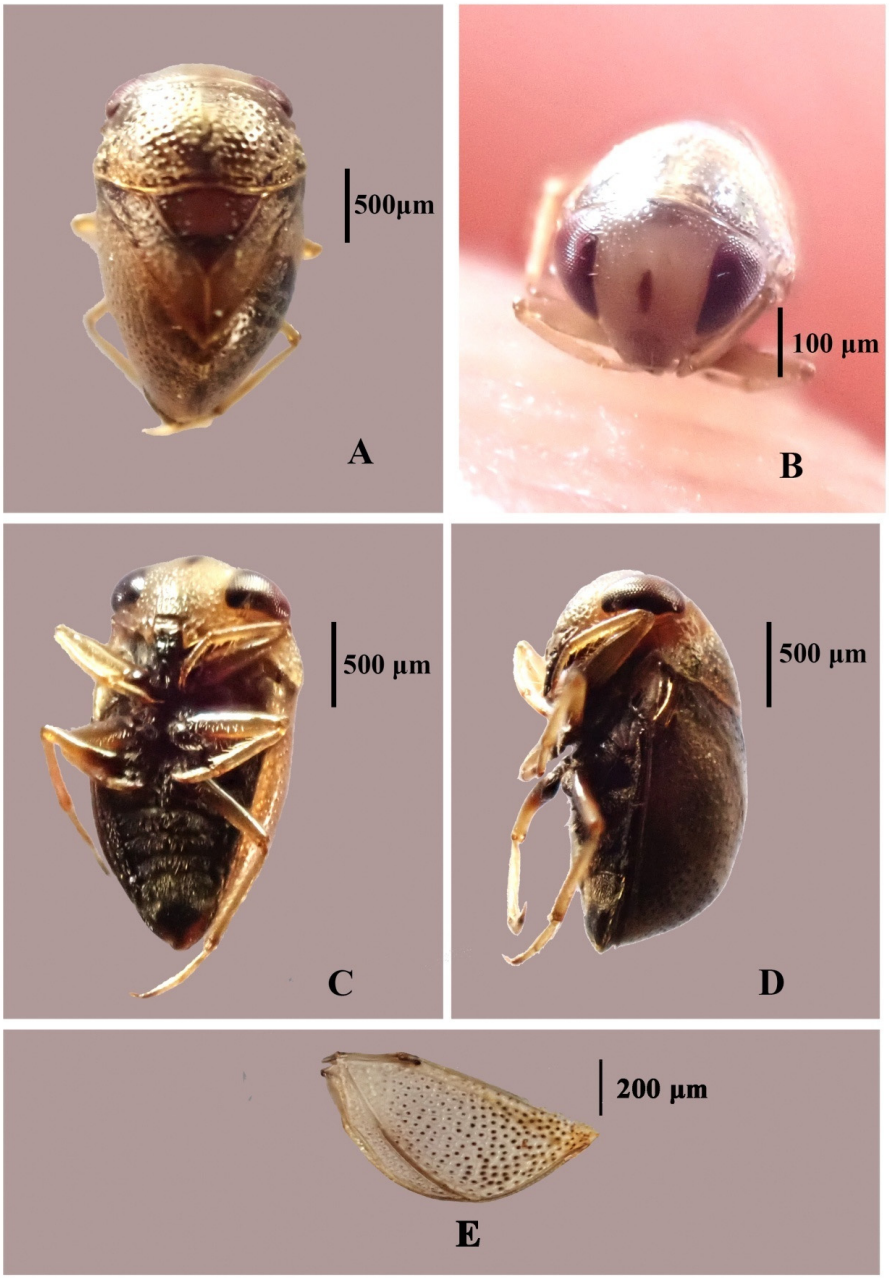


Fig. 1 A - E. *Paraplea frontalis* (Fieber, 1844) A. Dorsal view; B. Frontal view; C. Ventral view; D. Lateral view; E. Hemielytra

1994. *Paraplea liturata* (Fieber): Agarker *et al., Bionature* 14: 78.

Materials examined: 2 ♂, Sasthamkotta Lake; Punnakkadu Kadavu, Kollam district, Kerala, 8m amsl., 9°2' 41.27"N; 76°37' 44.17" E, 24.ii.2022, Coll. K. Jyothylakshmi and S. Nandakumar.

Diagnosis: Body size: 1.2- 1.6mm; colour: yellowish brown to golden brown; dark bands on hemelytra; eyes red coloured; antenna three-segmented, hidden below; punctures spread all over the body; five characteristic dark black spots on the pronotum; legs with numerous setae and tiny spines; thoracic regions clearly separate, anterior thoracic keel

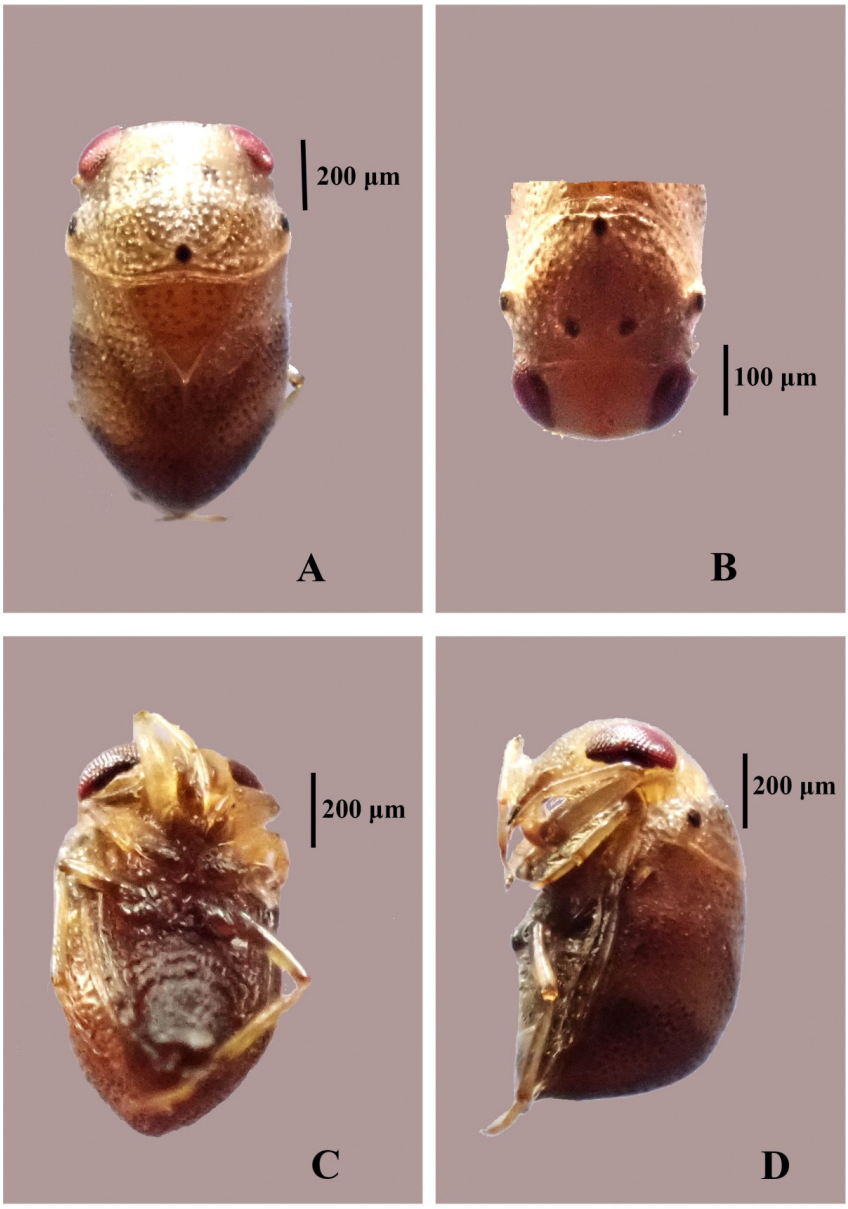


Fig. 2 A- D. *Paraplea liturata* (Fieber, 1844) A. Dorsal view; B. Head and Pronotum; C. Ventral view; D. Lateral view

rounded; two posterior thoracic segments serrated; abdominal keel with anterior two segments conjoined; male subgenital plate almost pentagon shaped, longer than wide, surface slightly corrugated; female ovipositor rectangle shaped with spines (Figs. 2A- D).

Distribution: India: Bihar, Delhi, Madhya Pradesh, Tripura and West Bengal

Bionomics: They were found crawling on the lake vegetation. Like other pleids, they prey on mosquito larvae and small invertebrates. The area in which the species was collected had a substantial amount of aquatic vegetation. The species is usually overlooked and rarely collected due to its minute size and swift movement. More comprehensive observations are needed to reveal their bioecology in detail, since only limited specimens have been

obtained during the present investigation.

Remarks: This is the first record of *Paraplea liturata* (Fieber, 1844) from Kerala. It can be easily separated from the closely resembling species, *Paraplea lateromaculata* Cook, 2020 by the presence of five distinct dark spots on pronotum.

A total of four species under the genus *Paraplea* such as *Paraplea buenoi* Kirkaldy, 1904, *Paraplea frontalis* (Fieber, 1844), *Paraplea indistinguenda* (Matsumura, 1905) and *Paraplea liturata* (Fieber, 1844) have been so far reported from India (Thirumalai, 2007). *Paraplea indistinguenda* (Matsumura, 1905) has been the only known species of *Paraplea* reported from Kerala so far (Thirumalai, 2007). The current record of *Paraplea frontalis* (Fieber, 1844) and *Paraplea liturata* (Fieber, 1844) is pivotal, since it is the pioneer report from Kerala and the records of the same from a wetland of international significance. The present faunistic records of the pygmy backswimmers are from Sasthamkotta lake, the largest freshwater lake of Kerala, and one among the top Ramsar sites of India. Macrophytes influence the diversity, abundance and distribution pattern of pleids by providing diverse ecological niches. Sasthamkotta Lake is infested with several native and non native macrophyte species. Therefore, it is imperative to conduct ample research activities on the taxonomy and faunistic of pygmy backswimmers of the lake, so that knowledge regarding this group can be utilized as baseline data for further research and conservation planning. *P. frontalis* (Fieber, 1844) was earlier reported from other states of India by Thirumalai (2004), Nieser *et al.* (2005), Thirumalai and Suresh Kumar (2005), Jehamalar and Chandra (2016) and Basu *et al.* (2018). Previous reports of *Paraplea liturata* (Fieber, 1844) were also made by Bal and Basu (2000) and Nahar (2004). Hence, keeping in view the scarcity of knowledge regarding the pygmy backswimmers of the State, intensive surveys in the unexplored ecosystems of Kerala are required. The present work could direct attention towards the necessity of faunistic study of pygmy backswimmers from other wetlands of Kerala to reinforce knowledge on its distribution.

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# *Calamus tenuis* Roxb. a new host for *Chilo suppressalis* (Walker) (Lepidoptera, Crambidae)

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**ABSTRACT:** *Calamus tenuis* Roxb. belonging to Arecaceae was recorded as a new larval host for *Chilo suppressalis* (Lepidoptera, Crambidae) from Kamrup district of Assam, India. However, other parts like leaf, stem, root and pistillate inflorescence were found unaffected.


**KEY WORDS:** Arecaceae, rattan, inflorescence, borer, Assam

Rattans are climbing palms of the monocot family Arecaceae (Renuka *et al*., 2010). The association between different insects and rattans has been reported by many workers in various countries (Chung, 1995; Mattes *et al*., 1998; Howard *et al*., 2001; Sunderland, 2004; Merklinger *et al*., 2014; Shameem and Prathapan, 2014; Liu *et al*., 2019). However, not much information is available on the insect pests of rattans in India (Renuka *et al*., 2010). *Calamus tenuis* Roxb. (Fig. 1 A) is one of the common and economically important rattan species of Assam. The staminate inflorescence of this dioecious species is around 3m long with 6-9 partial inflorescence (Mehmud and Roy, 2021). After anthesis (Fig. 1 B), the staminate inflorescences dry up and their remains were found to adhere to the plant for several months. During observations of the staminate flowers, larvae measuring 2-2.5cm (Fig. 1 C-E), were noticed on the rachis, which were reared (Fig. 1, F-I) and got identified as *Chilo suppressalis* (Walker) belonging to Lepidoptera, Crambidae. However, other parts like leaf, stem, root and pistillate inflorescence were found unaffected.

Khan *et al*. (1991) reported that *C. suppressalis* as an important rice borer in India, Indonesia and East Asia and there are around 41 host plants belonged to 30 genera under six families (four monocot and two dicot), where most of the plants were of the grass family Poaceae. Adult *C. suppressalis* measuring 1.3cm, is pale brown. Although *C. suppressalis* affects every part of the rice plant (Lu *et al*., 2017; Meng *et al*., 2019), it is worth mentioning that in rattans, only the staminate inflorescence of *C. tenuis* is infested. Voucher

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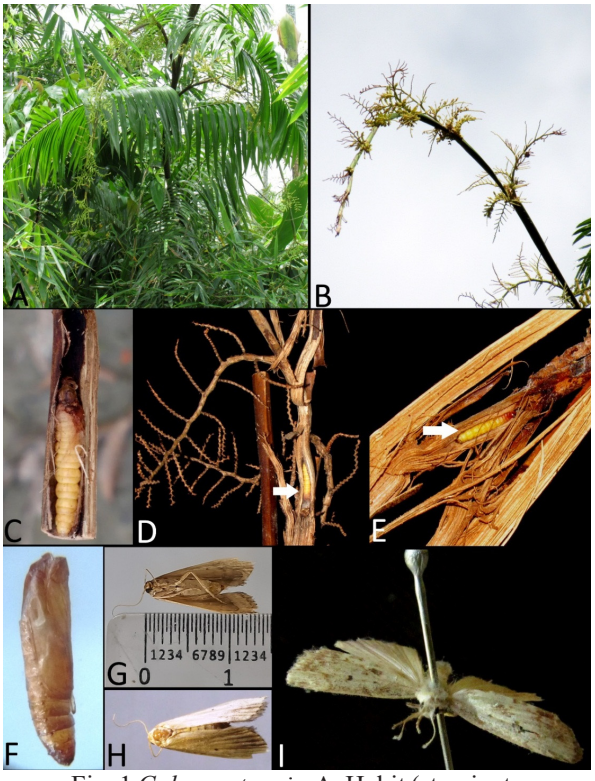


Fig. 1 *Calamus tenuis*: A. Habit (staminate inflorescence), B. Anthesis of staminate inflorescence. *Chilo suppressalis*: C. Larva inside rachis; D-E. Larva inside partial staminate inflorescence; F. Pupa; G-I. Adult.

specimens were submitted in the Department of Botany and *C. suppressalis* in the Department of Zoology, Handique Girls' College respectively.

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